Museum
QH
431
A2 A56

APRIL 1952

OF MICHIGAN
DEC 5 1952

Volume 6 Part 1

MENDEL DARWIN MORGAN

MUSEUMS
LIBRARY

HEREDITY

An International Journal of Genetics

Edited by

Cyril D Darlington DSc FRS
Hertford

Ronald A Fisher ScD FRS
Cambridge

in collaboration with

G W Beadle
Pasadena

T Caspersson
Stockholm

Th Dobzhansky
New York

B Ephrussi
Paris

Ø Winge
Copenhagen

Oliver and Boyd
London and Edinburgh

JOHANNSEN GALTON WEISMANN VILMORIN

VAVILOV LINNAEUS DE VRIES SPALLANZANI

BOVERI BATESON WILSON

INFORMATION FOR CONTRIBUTORS

Heredity publishes three times a year original articles of interest to research workers, teachers, and students in the fields of experimental breeding, cytology, statistical and biochemical genetics and evolutionary theory. New research, history review and criticism are accepted.

MANUSCRIPTS should be prepared so as to conform with the usage in current numbers of the Journal, especially with regard to Illustrations, References, Tables, and Summaries. The address of the *Author* and the Date of Receipt of the Manuscript will be printed under the *Title* which should be scrupulously abbreviated. The following abbreviations for References will be used :—

C.R.	*Comptes rendus*
J. Genet.	*Journal of Genetics*
P. Int. Gen. C. (7) . .	*Proceedings of the 7th International Genetics Congress*
P.N.A.S.	*Proceedings of the National Academy of Sciences, Washington*
P.R.S., B	*Proceedings of the Royal Society of London*, Series B
P.R.S. Edin., B . . .	*Proceedings of the Royal Society of Edinburgh*, Series B
Z.I.A.V.	*Zeitschrift für induktive Abstammungs- und Vererbungslehre*

TEXT FIGURES must be (1) suitable for line-block reproduction, (2) lettered in pencil, (3) supplied with legends and (4) separated from the text but allocated to sections of the text.

PLATES should be mounted to fit or exceed the whole size of the page ($10'' \times 6\frac{1}{4}''$) if possible. Half-tone plates can be bled if wished. Colour plates will be accepted only if the author bears half the cost of reproduction.

PROOF is supplied once, in Page form, together with an Order Form for Off-prints. Excessive corrections will be chargeable to the Author.

OFF-PRINTS are free to the number of 50. Additional copies will be supplied at cost if ordered before the issue is printed.

EDITORS :—

Dr C. D. DARLINGTON
John Innes Institution
Bayfordbury, Hertford, Herts.

Prof. R. A. FISHER
Whittingehame Lodge
Cambridge

Single Numbers, 21/- or $3.50 net
Annual Subscription, 60/- or $10.50 net

Printed and Published in Great Britain by OLIVER AND BOYD LTD.,
Edinburgh; Tweeddale Court. London; 98 Great Russell Street, W.C.

HEREDITY

An International Journal of Genetics

HEREDITY

An International Journal of Genetics

Edited by

Cyril D Darlington DSc FRS
London

Ronald A Fisher ScD FRS
Cambridge

Volume 6
1952

Oliver and Boyd
London and Edinburgh

Medical Library
610.5
H542
V.6

PRINTED IN GREAT BRITAIN BY
OLIVER AND BOYD LTD., EDINBURGH

Transfer to:
Medical
1-17-63

CONTENTS

PART 1. APRIL

PAGE

FISHER, R. A. (Cambridge). Statistical methods in genetics 1

MOURANT, A. E. (London), and WATKIN, I. MORGAN (Aberystwyth). Blood groups, anthropology and language in Wales and the Western Countries 13

STANTON, W. R. (Hertford). Bolting, a vegetative variation in the potato . 37

MULDAL, S. (Hertford). The chromosomes of the earthworms. I. The evolution of polyploidy 55

JINKS, J. L. (Birmingham). Heterocaryosis in wild *Penicillium* 77

REES, H. (Hertford and Birmingham). Asynapsis and spontaneous chromosome breakage in *Scilla* 89

DOWDESWELL, W. H. (Tiverton), and FORD, E. B. (Oxford). Geographical variation in the butterfly *Maniola jurtina* 99

RACE, R. R., and SANGER, R. (London). The inheritance of the *Duffy* blood groups 111

SÁNCHEZ-MONGE, E. (Zaragoza). The estimation of linkage with incomplete penetrance 121

COMMENT AND REVIEWS:

SAX, K., and LUIPPOLD, H. The effect of fractional X-ray dosage on the frequency of chromosome aberrations 127

LANE, G. R., on Sax and Luippold's fractionation experiment . . 131

DARLINGTON, C. D. on Cook's human fertility: the modern dilemma . 132

DARLINGTON, C. D. on Scientific thought in the twentieth century - 132

Books Received 133

GENETICAL SOCIETY OF GREAT BRITAIN: Abstracts of Papers . . . 134

PART 2. AUGUST

WYLIE, ANN P. (Hertford). The history of the garden Narcissi . . 137

SACHS, LEO (Cambridge and Hertford). Chromosome mosaics in experimental amphiploids in the *Triticinae* 157

PALMER, T. P. (Lincoln, N.Z.). Population and selection studies in a *Triticum* cross 171

COPP, L. G. L., and WRIGHT, G. M. (Lincoln, N.Z.). The inheritance of kernel weight in a *Triticum vulgare* cross 187

BILLINGHAM, R. E., LAMPKIN, G. H., MEDAWAR, P. B., and WILLIAMS, H. LL. (London and Edinburgh). Tolerance to homografts, twin diagnosis, and the freemartin condition in cattle 201

HOLT, H. A., THOMPSON, J. S., SANGER, R., and RACE, R. R. (London). Linkage relations of the blood group genes of man . . . . PAGE 213

CAIN, A. J., and SHEPPARD, P. M. (Oxford). The effects of natural selection on body colour in the land snail *Cepaea nemoralis* 217

SHEPPARD, P. M. (Oxford). Natural selection in two colonies of the polymorphic land snail, *Cepaea nemoralis* 233

SHEPPARD, P. M. (Oxford). A note on non-random mating in the moth *Panaxia dominula* (L.) 239

DAWSON, G. W. P. (Dublin). The frequency of the A B O blood groups in Dublin 243

SNELL, G. D. (Bar Harbour, Maine). Preliminary data on crossing-over between *H*-2 and *Fu*, *Ki* and *T* in the mouse 247

FALCONER, D. S. (Edinburgh). Location of " Reeler " in linkage Group III of the mouse 255

MAINLAND, G. B. (Auburn, Alabama). Epistatic ratios involving two phenotypes 259

WINGE, Ö. (Copenhagen). The genetic situation concerning fermentation in yeasts 263

COMMENT AND REVIEWS:

BAILEY, N. T. J., on Catcheside's Genetics of Micro-Organisms . . 271

PONTECORVO, G., on Genetics in the Twentieth Century . . . 273

MEDAWAR, P. B., on Darlington and Mather's Genes, Plants and People 274

LEWIS, D., on Morton's Soviet Genetics 276

Short Reviews 277

GENETICAL SOCIETY OF GREAT BRITAIN: Abstracts of Papers . . . 279

PART 3. DECEMBER

BATEMAN, A. J. (Hertford). Self-incompatibility systems in angiosperms. I. Theory 285

CAVALLI, L. L., and MACCACARO, G. A. (Cambridge). Polygenic inheritance of drug-resistance in the bacterium *Escherichia coli* 311

LAMOTTE, M. (Paris). Le rôle des fluctuations fortuites dans la diversité des populations naturelles de *Cepaea nemoralis* (L.) 333

SHARMAN, G. B., and BARBER, H. N. (Tasmania). Multiple sex-chromosomes in the marsupial, *Potorous* 345

SACHS, LEO (Cambridge and Hertford). Polyploid evolution and mammalian chromosomes 357

DOWRICK, G. J. (Hertford). The chromosomes of *Chrysanthemum*. I. The species 365

PAGE

HASKELL, GORDON (Hertford). Genetics of cold tolerance in maize and sweet corn seed 377

NELDER, J. A. Some genotypic frequencies and variance components occurring in biometrical genetics 387

SEARLE, A. G. (London). A lethal allele of dilute in the house mouse . . 395

OSTER, IRWIN I. (Bloomington, Indiana). A study of ultraviolet induced lethal mutations in *Drosophila melanogaster* 403

REVIEWS:

DARLINGTON, C. D. On Hughes' mitotic cycle 409

Short Reviews 409

INDEX 411

Marshall's
Physiology of Reproduction

Volume Two

Edited by A. S. PARKES, M.A., D.SC., SC.D., F.R.S.

Since the first appearance of this book in 1910 and its second edition in 1922, the subject has expanded enormously in almost every direction, and it has been found necessary to publish this third edition in two volumes, the work of the revision having been undertaken by a large panel of experts under the editorship of Dr A. S. Parkes.

The scope and general sequence of this standard work remains much the same as in the two previous editions, but practically the whole of the text has been rewritten and some new chapters added. Volume Two deals with embryonic and foetal development and physiology, lactation, and certain general problems. Volume One, which is in preparation, will deal with events up to and including fertilisation.

£7 : 10 : 0 net

LONGMANS

THE WORLD'S GREATEST BOOKSHOP

FOYLES

* FOR BOOKS *

FAMED FOR ITS EXCELLENT
MEDICAL BOOKS DEPARTMENT

New, secondhand and rare Books on every subject

Stock of over three million volumes

WE BUY BOOKS, STAMPS, COINS

119 - 125 Charing Cross Rd., London, W.C.2.

Gerrard 5660 (16 lines) ★ *Open 9—6 (including Saturday)*

ANIMAL BREEDING ABSTRACTS

THE JOURNAL THAT WILL KEEP YOU UP TO DATE WITH ANIMAL BREEDING IN ALL PARTS OF THE WORLD

In addition to Abstracts, Book Reviews, News and Notes, etc., each number contains a Review Article. Recent Articles include:

* The Heritability of Milk and Butterfat Yield, by Professor Ivar Johansson.

* Experimental Analysis of the Inheritance of Productivity and Growth in Pigs, by Dr J. M. Rendel.

† Research in Animal Breeding and Quantitative Genetics: a review of work in progress at the Institute of Animal Genetics, Edinburgh.

* Heteroploidy in Mammals, by R. A. Beatty.

* Operational Research, by Professor C. H. Waddington.

* Recent Trends in Animal Breeding Research in the U.S.S.R., by G. R. Ritchie (In press).

(Separates of these papers are available at *1/3 †1/6.)

Issued quarterly by the Commonwealth Bureau of Animal Breeding and Genetics, Edinburgh. Annual subscription: 35/- (28/- in the Commonwealth if ordered direct.)

Orders should be sent to: COMMONWEALTH AGRICULTURAL BUREAUX
CENTRAL SALES BRANCH
FARNHAM HOUSE
FARNHAM ROYAL
NEAR SLOUGH, BUCKS

Notes for Introductory Courses in Genetics

By

Charlotte Auerbach, Ph.D., D.Sc.

A concise account of the basic laws and concepts of genetics which should prove useful to workers in other branches of science as well as to students.

Price Two Shillings net

Oliver and Boyd
Edinburgh: Tweeddale Court
London: 98 Great Russell Street, W.C. 1

HEREDITY VOLUME 6 PART 1 APRIL 1952

STATISTICAL METHODS IN GENETICS

R. A. FISHER

Being the Bateson Lecture delivered at the John Innes Horticultural Institution on Friday, 6th July 1951 *

THE title chosen for this lecture is " Statistical Methods in Genetics," but after hearing what I have written you may think that it is not so much an account of the contributions which the study of Statistics has made to the advance of Genetics, as an examination of the nature of genetical Science from the point of view of a statistician. My reason for taking this point of view is that for many active years of my life I was fully engaged in doing what I could to further the progress of statistical methods, and that though for 25 years or so I have enjoyed the excitements of genetic experimentation, Genetics has only gradually come to be, for me, a whole time study, in place of a very fascinating hobby. Since that experience is in itself exceptional, it may be that the reflexions gathered on the way are worthy of record, and of interest to my genetical colleagues.

Genetics and Statistics have in common that they are both characteristic products of the twentieth century. As we should recognise them now, neither of them existed before the year 1900. Of course I do not ignore the fact that earlier workers of many nations, Naudin and Godron, for example, Knight, Gaertner and Kohlreuter broached the practical problems of experimentation in heredity, or that on the theoretical side Galton and Weismann put forward speculations sometimes to an interesting extent near the truth. The impression upon the modern reader of these earlier workers, and it is on this criterion that I base my claim, is that the writers, intelligent as they were, did not really know what they were writing about. They had not the concepts—the framework of ideas—in terms of which their thought might have become lucid and coherent. Mendel himself should, of course, be excepted, for he was framing these very concepts. Now just the same is true, in its own field, and without a parallel exception, of the writers on Statistics of the eighteenth and nineteenth centuries. A start had been made with the problem of gathering demographic data ; some beautiful mathematics, the theory of probability, had emerged in the study of games of chance ; the massive intellect of Gauss had brought order into the chaotic difficulties facing astronomers and surveyors in the combination of observations.

* This lecture has been established by the John Innes Trustees to be given at the conclusion of the biennial Summer Course in memory of William Bateson, first Balfour Professor of Genetics in the University of Cambridge and first Director of the John Innes Institution, who died twenty-five years ago, 8th February 1926.

Yet any one of the great names of the past, De Moivre or Bayes, Boole or Gauss himself, if by a miracle we can imagine him indoctrinated with the thought of our time, would, I believe, be astonished by the cogency and precision, the directness and accuracy with which problems formerly intolerably encumbered, can, in this age, be recognised, and resolved. In fact they lacked the concepts needed to think clearly about many of their problems.

Genetics and Statistics, then, have in common that each in its own field represents a distinctive point of view, which profoundly influences the intellectual processes with which scientific work is approached. I have during the past 15 years looked on, from a point of some advantage, at the process of the serologists, concerned with the human blood groups, becoming, as one may say, genotype-conscious. As this progress continues so, step by step does the worker's facility continuously sharpen in recognising the situation with which his observations confront him. What is even more striking is the confidence with which they now reach out to make new discoveries, which, without the genetic interpretation would never have been foreseen ; as is clearly shown by the cases in which these advances have been indignantly repudiated as impossible, until discovery had finally and firmly established them as fact. This, indeed, is exactly what happened with the antibodies anti-*d* and anti-*e* of the Rhesus blood-group system. By 1944 the existing testing fluids had been recognised to contain four different antibodies specifically related to four of the six genes by which the inheritance of this constitutional factor could be specified ; for two of them however, no specific antibody was known. It was theoretically possible that no such antibodies really existed. I and others received letters vehemently insisting that no such antibodies could possibly exist, for reasons which, however, carried no conviction, for any clear apprehension of the genetic system, revealed by tested relationships, certainly suggested that they were worth looking for. Before the end of that year indeed Mourant in England had discovered one of them, anti-*e*, in the serum of a patient suffering from a chronic anæmia. The important point is that he knew at once what he had discovered, and had no doubt of what tests to apply to confirm and establish his discovery. The second of the missing antibodies was more elusive ; Diamond at Harvard found the first example, but in such high dilution, and with such admixtures, that it could not be used with confidence. A year or two later Hill and Haberman were able to demonstrate it unmistakeably in sera from two Louisiana negresses. The confirmation of the genetic point of view afforded by Mourant was, it may well be thought, a necessary condition for the recognition of either the possibility or the practical importance of the second discovery to be sufficiently appreciated for it to be pursued with the confidence and tenacity needed to bring it to completion.

Only this year another antibody, this time one searched for long

and confidently, has been added to the serologists repertoire. The system in this case is that known as MN, first discovered in 1927 by Landsteiner and Levine using immune rabbit serum. In 1947 Race and Sanger showed that a new antibody, anti-S, in a serum from Australia subdivided each of the alleles M and N disproportionately, so that the antigen S with which it reacted was present in 25 out of the 53 per cent. M, but in only 8 of the 47 per cent. N in the English population. Clearly the new antigen was inherited almost if not quite at the same locus as M and N. With the example of the Rhesus antibodies in view, it was from the first anticipated that a fourth antibody, diagnostic in this system, must also exist, complementary to anti-S, and shortly after Easter this year I learned that the long search had been rewarded. The system *MNSs* with each of four genes recognisable by a separate antibody, is now the most powerful which we possess for such tasks of applied genetics as the exclusion of falsely ascribed paternity, or the recognition of interchange of the newly born in maternity hospitals.

	M	N	T
S	25	8	33
s	28	39	67
T	53	47	100

FIG. 1.—Frequencies of gene combination of the blood group factor *MNSs*.

That is only an example of the analytic penetration in biological enquiry supplied by a familiarity, not with an enormous range of genetical facts, but with primary genetical concepts, and that background which may be called the genetical point of view. A rather similar clarification must have taken place at the end of the eighteenth century with Lavoisier's formulation of chemical nomenclature. Something like it, though perhaps not so final, has occurred with the development of quantum mechanics ; but the really striking parallel of our own times is the emergence of modern statistics. Quite suddenly in the intellectual history of mankind it has become possible to think coherently and confidently about variation, a phenomenon which, when we reread nineteenth century authors, we see to have blocked and inhibited their thought to a degree which we find almost unimaginable. Apart from a universal catastrophe, we shall never think the same way again. The processes of interpretation which we apply to observational or experimental data are now capable of examination, not only rationally, but conclusively. Experimental design has become an *intelligible* subject for discussion, not merely by improvement in technique, but by a change in point of view. And here I may mention a connection between our two subjects which seem not to be altogether accidental, namely that the "factorial" method of experimentation, now of lively concern so far afield as the psychologists, or the industrial chemists, derives its structure, and its name, from the simultaneous inheritance of Mendelian factors. Geneticists certainly need not feel that the intellectual debt is all on one side.

A new subject for investigation will find itself opposed by indifference, by inertia, and usually by ridicule. A new point of view, however, affecting thought on a wide range of topics may expect a much fiercer antagonism. *Amour propre* is deeply aroused. Unproductive minds,—and God knows it is by no fault of their own that they are so—who by long occupancy of a rostrum have come to think of themselves as authorities, cannot easily brook the idea that they must *reconsider* their opinions. They have staked so much on their opinions being beyond question. Human nature being what it is, it is not surprising that such subjects encounter educational difficulties, and are not easily welcomed as educational disciplines, and this for the very reason which makes them educationally essential for a generation which is to live in the twentieth century, namely that they can instil the conceptional backgrounds upon which modern points of view have been established.

As the position is a serious one, and its educational importance, I believe, enormous, I do not want to give any false impression. It is not a simple matter of the jealous ignoration and exclusion of a rival field of study. That phase in a sense, has been passed. The importance of Genetics, as of Statistics, is recognised ; at least lip-service must be paid to it. At the present time there are botanical departments which like to make a show of teaching Genetics ; they like it better if it can be taught as a subordinate branch of systematic Botany. There are zoologists, too, who sometimes use the language of Genetics. Some of these, however, think that it is a part of the physiology of reproduction ; others that it is an aspect of experimental embryology. On these terms what they know of the subject can be accommodated to their outlook, and students anxious to learn something of Genetics can be diverted to other interests. We need not believe that such misrepresentation is consciously fraudulent. It is sufficiently explained by ignorance of what Genetics is about, and ignorance of the means that must be taken to become acquainted with the subject. Obviously, also, Genetics can and does contribute to knowledge in these other fields, but to say this is very different from saying that a study of these fields will supply, in any degree, that particular kind of understanding which it is the object of genetic studies to attain.

Statistics, it should be noted, has encountered parallel difficulties in education. The large mathematical departments, with no tradition of living contact with experimental situations, have attempted to accommodate statistical teaching as an eccentric kind of pure mathematics. Quite a number of books with statistical titles have appeared recently, especially in the United States, which show clearly the difficulty of grafting the ideas, which alone make statistics fruitful, on to a purely deductive mathematical tradition. To many, evidently, the existence of Statistics can be comfortably recognised if, and only if, it can be regarded as an application of the Theory of Probability, a

theory which, in my innocence 30 years ago, I had thought to have been absorbed and partly superseded by the development of statistical ideas. In Paris a few years ago I was delighted to learn that the mathematicians of the Sorbonne were, with truly Gallic insight, drawing a distinction between "les probabilists" and "les statisticiens." Statistics evidently has a hard task in the atmosphere of an old mathematical department not to be changed back into something more conformable to the nineteenth century. Solid progress is indeed being made, but it is at places like Rothamsted in this country, or in the Bureau of Standards in America, rather than in places organised primarily for education.

Now, if I am right as to the inevitability of the obstacles put in the way of new disciplines, and especially of those from which we have most to gain, we can begin to appreciate the great part played by William Bateson in consolidating Genetics as an independent stream of thought, in this country, and throughout the world. We can appreciate too the wisdom of that most significant step in his career when he vacated the chair of Genetics at Cambridge, to tend and establish the nascent Institution founded by John Innes. Genetics needed a champion, and Bateson did not hesitate to recognise that he was cast for that role. Very quickly he had rejuvenated the Evolution Committee of the Royal Society, and, as the reports of that body show, had formed a group of enthusiastic workers who, in the first few years of the century, had already verified and considerably extended the Mendelian doctrine.

It is said that Bateson had no liking for statistics; and for the half-bogus statistical arguments by which that forensic adept and indefatigable controversialist, Karl Pearson, had tried to snuff out the discovery of Mendel's work, he had no reason for feeling either liking or respect. I do not think that he knew much about statistics, or that the subject seriously occupied his attention, but that he was essentially unprejudiced, the following example will show. In 1909 he published his *Mendel's Principles of Heredity*, a book that I bought that year as a mathematical freshman. It includes a translation of Mendel's paper on "Pisum." Criticising the work on inheritance of his German predecessors, Mendel says (p. 318) :—

"Those who survey the work done in this department will arrive at the conviction that among all the numerous experiments made, not one has been carried out to such an extent and in such a way as to make it possible to determine the number of different forms under which the offspring of hybrids appear, or to arrange these forms with certainty according to their separate generations, or definitely to ascertain their statistical relations."

Bateson takes the opportunity to add the following significant footnote :—

"It is to the clear conception of these three primary necessities

that the whole success of Mendel's work is due. So far as I know this conception was absolutely new in his day."

In 1909 Bateson had won his campaign, on the scientific front. On the academic front he had perhaps reconnoitred the position. Whatever may have been his reflections, it is clear that they led him to conclude that it was in the Research Institution rather than in the University Department that Genetics could grow to its full stature. He may have felt that he himself had something better to do than to compete, in an atmosphere of rather feline jealousy, with some one or other somewhat decayed branch of traditional Zoology.

I do not know how much foresight to ascribe to Bateson in this matter. Looking back it is, I think, clear to all of us that his choice was wise, and in itself constituted a major contribution to the progress of Genetics. Obviously, the conditions for teaching the subject in Cambridge would now have been better if Bateson had sacrificed the best years of his life, all too short as it proved to be, to grappling with the obstacles, which would have been thrown in his way. In principle, at least, I am glad that the John Innes bequest offered an alternative to such a sacrifice.

One great advantage of Genetics over the earlier biological studies is that it brings the research worker into close contact with living material. It should occupy his attention, and be under his constant supervision. As Bateson so often insisted the geneticists laboratory is the garden plot, or the breeding pen. It is not, I believe, sufficiently realised that this need for absolute realism is particularly required in statistical work when applied to genetic purposes. It is in general the statisticians task to bring theory into a truly organic coherence with objective and verifiable observations. Genetics is almost alone among the biological sciences in having a definite and compact, though doubtless imperfect, theoretical basis. Its characteristic frequencies are a constant stimulus to statistical enquiry. An important step was taken towards making Statistics competent to aid in Genetics, when the theory of small samples was developed. So long as the statistician was supposed to concern himself only with vast aggregates of massed data, his acquaintance with the detailed processes by which they came into existence was bound to be vague ; each part of the whole contained its own complications and its own enigmas ; in the treatment of the mass these were necessarily almost wholly unrecognisable, and the interpretation was harassed by innumerable unanswerable queries. As soon, however, as it was realised that small bodies of data, such as the progeny of a single mating, could be examined by competent and exact methods, the particular circumstances of each case could be considered, the homogeneity of different groups examined, and generally speaking, a check could be put to the process by which good data are contaminated by bad.

Direct contact with what is actually done in experimentation

helps the statistician in another very essential way, by leading him to consider variations in procedure, and the reasons why one method is to be preferred to others. The whole wide subject of experimental design is opened out by this consideration. Genetics is indeed in a peculiarly favoured condition in that Providence has shielded the geneticist from many of the difficulties of a reliably controlled comparison. The different genotypes possible from the same mating have been beautifully randomised by the meiotic process. A more perfect control of conditions is scarcely possible, than that of different genotypes appearing in the same litter. Generally speaking the geneticist, even if he foolishly wanted to, could not introduce systematic errors into the comparison of genotypes, because for most of the relevant time he has not yet recognised them. What a beautifully controlled experiment, again, is put into the geneticist's hands in a linkage test by simple backcross to a multiple recessive. Here each pair of allelomorphic genes should occur, though in different combinations, equally in each of the pairs of complementary genotypes which constitute his results. The first step to perfect control against viability disturbances, is then inherent in his material.

Spoiled favourites of the gods, as in this matter they are, geneticists have been in some cases a little slow to make use of experimental precautions, which in other fields have been found to be necessary. Some years ago, in the early days of quantitative studies, it was quite usual for an investigator of a wide varietal cross to provide data showing that the F_2 was more variable than the F_1. Of course, this was very probably true ; and if true it was certainly of genetic significance. But an F_1 of perhaps 50 plants carefully grown on a small uniform plot is not cursorily to be compared with an F_2 of 5000, set out on a hundred times the area. The geneticists of those days, however, showed no awareness that even clonal material, compared in this way would probably have shown some difference in variability in the direction observed. Their humble colleagues in farm institutes had no doubt heard of soil heterogeneity, and the more intelligent of them could perhaps have suggested how an unbiassed comparison could have been made, but, at that time, such knowledge was not expected of geneticists. Indeed to this day, I would not know where to turn up, in an elementary textbook of Genetics, where surely it belongs, an account of how such a comparison could properly be made.

That example, although exceedingly simple, may be said to belong to the field of biometrical Genetics, where statistical ideas are admittedly essential. In linkage studies, however, the same point may be made. I mentioned further back how well controlled a linkage backcross was from disturbances due to unequal viability. Well controlled, but not perfectly controlled, for differential viability due to interaction of two or more factors will still, in a single cross, disturb the recombination fractions. Here the gods have provided

the remedy, in that the test can be carried out in two ways, in coupling, or in repulsion. Using both tests the effects of differential viability can be measured and eliminated. In three-point tests, moreover, where four modes of gamete formation can be recognised, there are four types of triple heterozygote available; and in four-point tests there are eight. By no merit of ours, indeed, the means are always available for making a clean evaluation. But very seldom have the means been used. In the literature of *Drosophila*, I have found a few cases of a complete three-point test. They are very bad. I doubt if a four-point test has ever been made in all eight ways. In the examples I have found, indeed, the purpose of the procedure has been gravely misunderstood, and the counts merely added up, in the *naïve* expectation that $ax+by$ is equivalent to $ay+bx$! Admirable as the early work with *Drosophila* was, the leading drosophilists of the United States were quite singularly unaware that anything was to be learned from statistical methods. Some seem to have cultivated the exceedingly misleading doctrine that with large enough numbers there was no need for critical experimentation.

Mode of gamete formation.

Genotye of parent	None	(aA)	(bB)	(cC)
+/abc	P	Q	R	S
aBC/Abc	Q	P	S	R
AbC/aBc	R	S	P	Q
ABc/abC	S	R	Q	P

FIG. 2.—Complete three-point test using all four triple heterozygotes. Column headings show which, if any, gene pairs are interchanged. Four pairs of genotypes of offspring are represented by the letters P, Q, R, S.

I mentioned just now the numbers of modes of gamete formation in a multiple backcross and the numbers, which happened to be the same, of multiple heterozygotes which could be used for breeding. What I said was obvious enough to those familiar to this type of work, yet I find that students are often surprised at finding that Genetics requires a recognition of combinatorial relationships. Yet from Mendel's paper to our own day, the primary distinctions of the language of geneticists have been combinatorial. In using terms like homozygote and heterozygote we are intuitively recognising that the number 2 may be made up of like parts, or of unlike parts. I believe this combinatorial aspect can usefully be brought to the attention of students, and as soon as the theory of polysomic inheritance is touched on it becomes a sheet anchor. In tetrasomic organisms instead of two kinds of organism defined by the partition of the number 2, we have five kinds defined by the partitions of the number 4, and in hexasomics there are 11, only one of which is homogenic, corresponding with the homozygote, while 10 correspond, but in considerably different ways, to the heterozygote, and contain various numbers of different allelomorphs.

In linkage studies with two loci disomic organisms present only

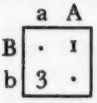
two doubly heterozygous types, distinguished as in coupling and in repulsion, which exhibit the effects of linkage in their gametic series. These two types are equivalent, or isomorphic, in their behaviour, since the gametic series of one is transformed into that of the other by a mere transposition of classes. With two factors in an auto-tetraploid the number of genotypes capable of exhibiting linkage effects is not two but no less than two hundred and sixty-six, but these may be recognised to fall into only twenty-four isomorphic sets, each capable of supplying its own peculiar information as to the linkage situation. The recognition of these sets is thus the first step to classifying an otherwise chaotic mass of possibilities. The results of using plants of any one set require to be supplemented by the results of using others.

In disomic work with two factors, there are only two modes of gamete formation which can be distinguished, the recombinants and the non-recombinants. With tetrasomic inheritance eleven modes are distinguishable. The frequencies with which these occur must be determined experimentally. When their values are more familiar, we may be able to recognise on what features of the meiotic process, and of the positions of the loci, they depend. Three features are easily recognisable, the frequency of recombinant strands, and the two frequencies of " double reduction," characteristic of the two loci. The observable frequencies do not specify the frequency of quadrivalent formation, nor enable us to compare exactly the frequencies of recombination in bivalents and quadrivalents. They do, however, supply an indication of the position of the centromere relative to the loci marked. Speaking of the future, the existence of eleven distinct modes of gamete formation, having frequencies capable of evaluation by direct breeding experiments, will certainly throw light from a new aspect on the geometry and mechanics of the meiotic process, apart from their primary naturalistic function of predicting the gametic output.

The experimental determination of these frequencies is not easy. Whereas in disomic material a simple backcross to a multiple recessive will serve to determine the genotype of each gamete that has been effective, the corresponding diagnosis of the diploid gametes of a tetraploid, or of the triploid gametes of a hexaploid requires successive backcrosses in two generations. Such work is relatively costly in space and time, but not extravagantly so. I grow every year rather more plants than did Gregor Mendel, but not three times as many. I am an advocate of economy in experimentation, but not of the cramping dogma that everything should be done with a few milk bottles, or petri-dishes.

A circumstance much more baffling than the need for a double backcross comes into view when we observe that having determined the genotypes of a sample of gametes, we have not thereby determined the mode of gamete formation by which each arose. Not only does

each mode of gamete formation give rise to several different genotypes, just as in disomic forms complementary pairs of genotypes have a similar origin, but, at the same time, the same genotype of gametes may be produced in more than one way. The relationship between the genotypes of the gametes formed and their possible modes of formation, is somewhat more complex than that to which we are used in disomic inheritance. In place of a simple series, we have an array of series, or what mathematicians call a matrix. The relation between the modes of gamete formation and the genotypes to which they give rise is established by an essentially simple, though sometimes tedious operation known as matrix multiplication. Now, let me not be



	Number of genotypes	Rank of matrix
Bisimplex coupling	4	8

Bisimplex repulsion	4	8

Simplo-duplex	4, 4	7, 7

Biduplex coupling and repulsion	2	6

Biduplex neutral	1	4

FIG. 3.—Six isomorphic sets of doubly heterogenic genotypes.

accused of introducing matrix algebra into Genetics, by a wanton act of highbrow sophistication. Rather consider *Lythrum salicaria* as making me recognise that its genetic operations may be compendiously mapped only by setting out the 24 gametic matrices corresponding with its 24 sets of isomorphic genotypes.

Of these 24 only 6 are available unless more than two alleles are available at the same locus. The simplest and most fundamental properties of these matrices are naturally of direct genetic concern. For example the rank of each matrix tells us the number of independent equations which experimentation can supply towards determining the eleven unknown frequencies of the modes of gamete formation. One has rank 4, one has rank 6, two have rank 7 and two rank 8. Each set of genotypes by itself can only supply partial information. Hence

the need for experimentation with genotypes of more than one isomorphic set. By combining data from two different sets I have brought the rank up to 9. With a third set I hope to bring it up to 10. The one piece of information that remains is inaccessible, unless three alleles can be mobilised at both of the two linked loci.

Now, I have retailed these details from my own work merely to underline and emphasise a general fact relevant to the present status of Genetics, namely that we shall not understand the process of inheritance as it occurs in nature unless we follow Mendel's example, and endeavour as he says " to determine the number of different forms under which the offspring of hybrids appear," and to that end extend, so far as our material requires it, those simpler combinatorial concepts which Mendel himself applied to the factorial system of unlinked factors in diploids. Of course, one can understand the academic mind seeking for problems of Ph.D. magnitude, and for material making the most modest demands upon space or time. After all the purpose of the Ph.D. thesis is to exhibit such competence as should be acquired in a successful apprenticeship. The limitations of relevance, and the monotony of ideas are proverbial. One does not want to go on doing that sort of thing all one's life. Moreover, the advancement of our science demands that we should not. Man-size problems are not hard to find. From the theoretical point of view it would be intolerable if we were unable to understand the process of inheritance in, for example, the cultivated potato ; while on the applied side the art of potato breeding will owe little to genetical science so long as geneticists have no experience of following segregation in that species, of investigating the peculiar genetical situations it presents, or of carrying out manipulative breeding projects with this difficult material.

The potential value of Genetics to the human race, lies in the enormous variety of genotypes which can be compounded by the combination of relatively few, though absolutely numerous, elements. In this it is in a position strikingly similar to that of Chemistry. Our elements are genes, and our compounds genotypes. The extension of interest to polysomic inheritance is in this analogy parallel to the extension which took place in Chemistry when the so-called " organic " compounds with their covalent bonds began to occupy attention, alongside the older Chemistry of acids and bases. Organic Chemistry looked at first intolerably complicated, and many of the older simplifications broke down. Yet its study, however discouraging at first, has certainly been rewarding, and I do not doubt that in a later generation experts in the manipulation of polysomic forms will be astonished at our lack of curiosity in exploring the new field, and at our lack of enterprise in exploiting it.

For the future of Genetics we may, I think, gain some hints from the way Chemistry has developed, for as a science with an effective theoretical basis it has a start of about a century over Genetics. In

that time the chemists have been able to provide themselves with a great variety of reagents, purified materials the reactions of which are largely known. Without them the chemist would be unable to recognise the chemical entities with which he has to deal, to examine what goes on in a chemical process, or to synthesise desired compounds. The geneticist has exactly parallel needs. We are aware of the need for multiple recessives, and for inbred lines as tools with which to probe an obscure genetical situation, but in the present state of our science we usually have to set about making the tools before we can use them. We do not find much already made. In examining the fifth linkage group of the house-mouse I spent 9 generations in making up a quadruple recessive between closely linked factors. This was not in the least extravagant, but, on the contrary, a very profitable investment. Such preparation work, involving a programme of preliminary matings, should be more general, and should occupy a larger proportion of a department's resources than is generally recognised. In a teaching department, to have such work going on is a first necessity, if anything like instruction in practical Genetics is to be attempted, for the preparation of genetic material needs both understanding and experience. I should suggest, too, that the continuity of policy which alone is possible at a great Institution devoted to genetic research, the routine analysis which is perfectly suitably carried out by trainees should be supplemented and supported by a permanent policy of preserving and enriching the collection of available genes and of reliably true breeding strains, the reactions of which are well known from previous experience.

Well that perhaps is as much as I can hope to put into an hour's discourse. We are the heirs of a tradition of potent ideas. The future of our science is full of promise. The more we reflect on the living creation with these ideas in mind the more there appears to be that can hopefully and fruitfully be attempted. Every generation in our science has the task of developing more penetrating and effective breeding techniques, for investigation, and for development. The full promise of our growing science can only be realised as we study these techniques, and apply them to those many problems which will only yield to professionally trained skill and experience. To preserve every point gained, and to enrich our armoury of resources, is especially the task of the permanent research establishments ; and it is for his foresight in accepting the directorship of John Innes' foundation that geneticists will always honour the name of William Bateson.

BLOOD GROUPS, ANTHROPOLOGY AND LANGUAGE IN WALES AND THE WESTERN COUNTRIES

A. E. MOURANT

Blood Group Reference Laboratory (Medical Research Council), Lister Institute for Preventive Medicine, London, S.W. 1

and

I. MORGAN WATKIN *

John Innes Institution and National Blood Transfusion Service, Wales

Received . .

1. INTRODUCTION

Our knowledge of the topographical distribution of the *ABO* groups in Europe is sufficient to enable us to draw a clear picture which is unlikely to be altered fundamentally by more detailed investigations carried out in the future. The distribution of the *Rh* groups is much less completely determined. On the whole the observations on these two blood group systems are readily reconciled with one another but certain apparent discrepancies need further investigation.

The object of this paper is to survey the available data as to the *ABO* and *Rh* blood group frequencies of the peoples of Western Europe, especially Wales, and of North Africa, and to suggest certain correlations between the distribution of the blood groups and the findings of historians, prehistorians and philologists.

The survey of the blood groups of the area, apart from Wales, is the work of A. E. Mourant. The entire study of the people of Wales and their relations with other populations and the conclusions are the work of I. Morgan Watkin.

2. BLOOD GROUPS

(i) *ABO*

General.—Europe can broadly be divided into three areas on the basis of the frequencies of the *ABO* blood groups. Most of Western Europe shows a high frequency of group *A* (as compared with the rest of the world) with medium *O* and rather low *B*, the percentage gene frequencies being of the order : $A = 30$; $B = 5$; $O = 65$. East of the Baltic and Adriatic seas and of a line running through Central Germany there is a rise of *B*, mainly at the expense of *O*, and this rise apparently continues steadily to give very high *B* levels in Central Asia and India.

West of the main area of high *A*, and at a number of other places on the periphery of Europe, we find very high *O* levels with *B*

* Working with the assistance of a grant from the Nuffield Foundation.

somewhat higher than in the high *A* area. These remarks apply to Iceland, Scotland and Ireland, to much of Wales and, to a less marked degree, to Northern England.

The *A*, *B* and *O* isogenes of the two western zones are shown in figs. 1-3 which are based on work described in this paper, on the compilations of Boyd (1939) and de Hoyos Sainz (1947), on an unpublished survey of the north of Scotland by Dr H. J. R. Kirkpatrick, and on a number of papers on individual countries, cited in the list of references.

Wales.—The inhabitants of Caernarvonshire, Denbighshire and Flint, particularly those bearing North Welsh surnames, have very high *O* frequencies. The figures given by Fraser Roberts correspond to percentage gene frequencies of $A = 19{\cdot}1$; $B = 5{\cdot}0$; $O = 76{\cdot}0$. *vide* figs. 4-6.*

In Merioneth along the coastal plain of Ardudwy which stretches from Barmouth to a point slightly north of Harlech, and in the small peninsula around Penrhyndeudraeth, the natives display a similar character. In the mountainous region of the Rhinog and Arennig to the east, however, the *O* frequency drops slightly and, in places, appreciably. Southwards from Barmouth, along the shores of Cardigan Bay through Towyn, Aberdovey and Aberystwyth, the *O* levels are still high.

There may be an island of high *O* on and around the Prescelly mountains in Pembroke but further data are needed in order to establish this.

In South Wales the Black Mountains of Brecknock and the adjacent Wye Valley stand out among their neighbours—the Herefordshire plain to the east and the Radnor Forest to the north—in harbouring a population of high *O* value.

A few small mountain areas show high *B* frequencies which are almost unique in Western Europe. They are discussed in detail in the section on anthropometry.

Speaking very generally, one might say that in South Wales only one or two regions display really high *O* levels, whereas in North Wales this feature is exhibited by the inhabitants of both hill and dale. This suggests that North Wales has not been subjected to the human migrations which have affected the southern half of the country.

* *Note.*—In compiling the gene frequency maps of Wales and the Marches, use was made of the Fraser Roberts (1942) figures for Caernarvonshire, Denbighshire and Flintshire and of additional data relating to these counties which is now available. The frequencies in the remaining areas are based entirely on unpublished data and relate to approx. 20,000 persons. Gloucestershire and Somerset (Fraser Roberts, 1948) have been included for comparative purposes.

The coalfields and the principal cities of South Wales have been excluded as their present-day inhabitants are not representative of the original populations. Glamorganshire, *e.g.* received between 1861 and 1911 400,000 immigrants from outside the county, more than half of whom hailed from beyond Wales. Monmouthshire received an even greater proportion of English immigrants.

Two regions supposed to be racially allied to Wales are Cornwall and Brittany. The former does not, however, differ significantly

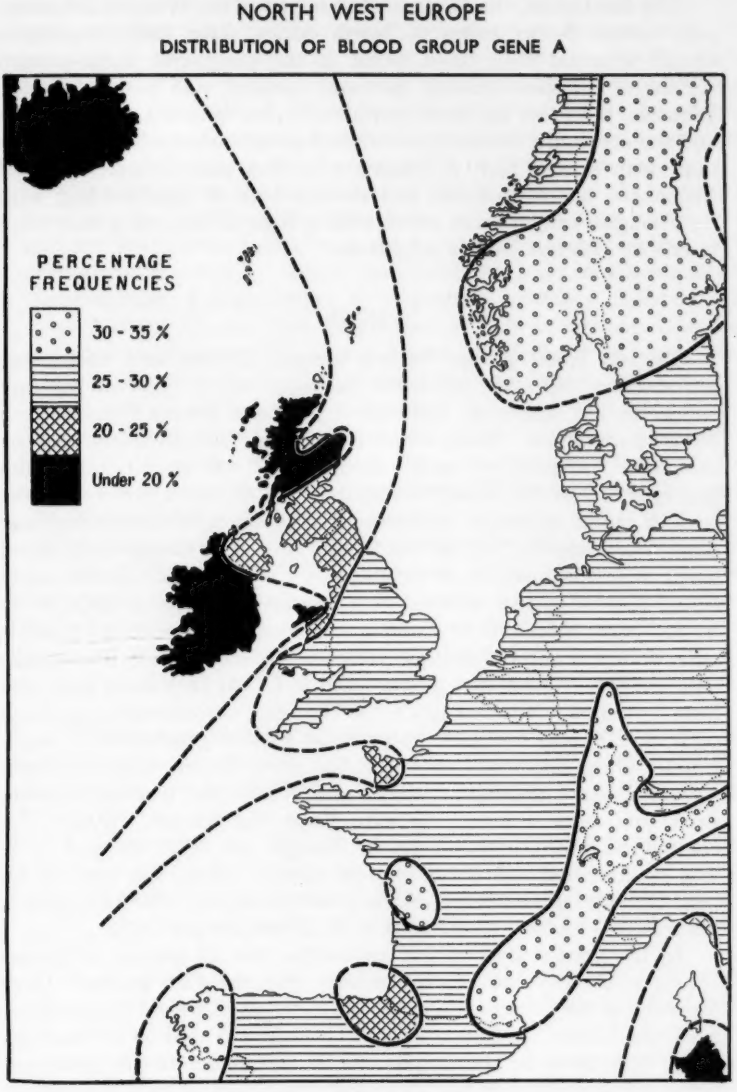


FIG. 1.

from the rest of Southern England whilst Brittany, as shown by Vallois, (1949) has *B* levels but not *O* levels higher than in the rest of France.

Special regions.—The Basques of South-western France and Northern Spain have a high *O* frequency together with the lowest *B* frequency in Europe by a considerable margin.

The Sardinians, the Cretans, the peoples of the Western Caucasus, and certain Berber tribes of North Africa show *ABO* frequencies almost identical with those found in the peripheral north-western regions of Europe—Iceland, Scotland, Ireland, and parts of Wales. Whereas, however, the latter populations live between a low *B* area and the ocean, the Caucasians and Berbers are surrounded by populations with a very high *B* frequency so that their relatively low *B* frequencies are remarkable and show a lack of interbreeding with their neighbours. Berber tribes with a high *B* frequency may have interbred with non-Berber neighbours.

(ii) *Rh*

The *Rh* blood groups form a complex system and need some introduction, especially since the available sets of observations vary widely in the degree of their serological and genetical subdivision of the population. Many surveys divide individuals solely into the categories "*Rh*-positive" and "*Rh*-negative." *Rh*-positives are of the genotype *DD* or *Dd*, while *Rh*-negatives are *dd*. The presence of the gene *D* in the genotype determines the presence of the serologically detectable antigen *D* on the red blood cells. The complete *Rh* blood group system is, however, determined by a set of genes at three closely linked loci on a chromosome, which may be occupied respectively by the genes *C* or *c*, *D* or *d* and *E* or *e*, each gene (with the possible exception of *d*) determining the presence of a serologically distinguishable antigen. Other rare alleles (such as C^w) at each locus have also been described. The linkages are so close that chromosome complexes such as *CDe* may for most purposes be treated genetically as single genes. Only one crossover, and that in a father, has ever been described but a deletion of the *C* and *E* loci (the true order being *DCE*) has been detected (Selwyn, Race and Sanger, 1950). The commonest gene combinations in Europe are *CDe*, *cde* and *cDE*. The combination *cDe* is common in Africa. Most, but not all, *D*-negative (*dd*) individuals are of the genotype *cde/cde*. For fuller details the reader is referred to the work of Race and Sanger (1950).

In the present state of our knowledge, the *Rh* groups of Europe appear to show a simpler arrangement than the *ABO* groups. Over the whole of the Continent north of the Pyrenees, Alps and Carpathians, from the Atlantic to Latvia and the Ukraine, as well as in Northern Italy, differences in the frequencies of the numerous *Rh* genotypes between one population and another seldom reach the level of statistical significance. Individuals who are *D*-negative (genotype *dd*) show a frequency never far from 16 per cent., indicating a gene frequency of 0·40 for *d* and 0·60 for *D*. The approximate frequencies of the

principal *Rh* chromosomes are *CDe* (including $C^{w}De$) 0·40 ; *cde*, 0·38 ; *cDE*, 0·16.

The Basques have by far the highest frequency of *D*-negatives of any known population in the world, in the neighbourhood of 30 per cent., together with the lowest *cDE* frequency in Europe (*CDe*, 38 per cent. ; *cde*, 53 per cent. ; *cDE*, 7 per cent.). Similar high frequencies of *D* negatives have been found among the neighbouring Béarnais and in Galician (N.W. Spanish) emigrants to the Argentine, but the number of Béarnais examined is small and specimens collected in Galicia itself show a very low frequency of *D*-negatives.

Observations on the frequencies of the *Rh* groups in the countries bordering the Mediterranean Sea have, up to now, been rather few and the frequencies found have shown wide variations, making any generalisation difficult. Apart from Northern Italy which resembles North-western Europe, there is a general tendency towards low frequencies of *cde* and high frequencies of *CDe*, as instanced by the populations of Ferrara (an isolated part of Northern Italy), several towns in Southern Italy, of Sardinia (Morganti, Panella and Cresseri, 1950), and of Galicia (Agosti Romero, Ikin and Mourant, 1950). The *Rh* chromosome frequencies rather closely resemble those found in India. Catalonia (Race *et al.*, 1949) shows high values both for *cde* and *CDe* suggesting a mixing of this Mediterranean type with the Basque stock. A distinct subtype is found in Egypt and Iraq (Kayssi, 1949) with a raised *cDe* frequency suggesting Negro influence.

The observations of Chown, Peterson, Lewis and Hall (1949) on European Jewish immigrants in Canada show that they differ appreciably from all the other peoples of Northern Europe, and suggest a mixing of North European and East Mediterranean components.

(iii) *General*

Having examined the distribution of the *ABO* and of the *Rh* groups we must consider how far these distributions are correlated both with one another and with the findings of anthropologists generally.

It is somewhat surprising to find all over Europe north of the great mountain ranges an almost completely even distribution of *Rh* groups coupled with wide variations of *ABO*, but the two categories do not in fact cut across one another as they appear to do in the Mediterranean area.

In the Basques we find the two systems agreeing fully in demonstrating that this population is one which has mixed very little with its neighbours. While, however, the existing Basques have thus remained unmixed, it is highly probable, as Chalmers, Ikin, and Mourant (1949) have suggested, that a people akin to the Basques once occupied a large part of Europe, and, mixing with mainly *D*-positive invaders, was the main source of the *d* gene throughout the continent. This suggestion agrees with the long-known physical

and cultural uniqueness of the Basques and with the fact that Basque place-names extend far beyond the present Basque country.

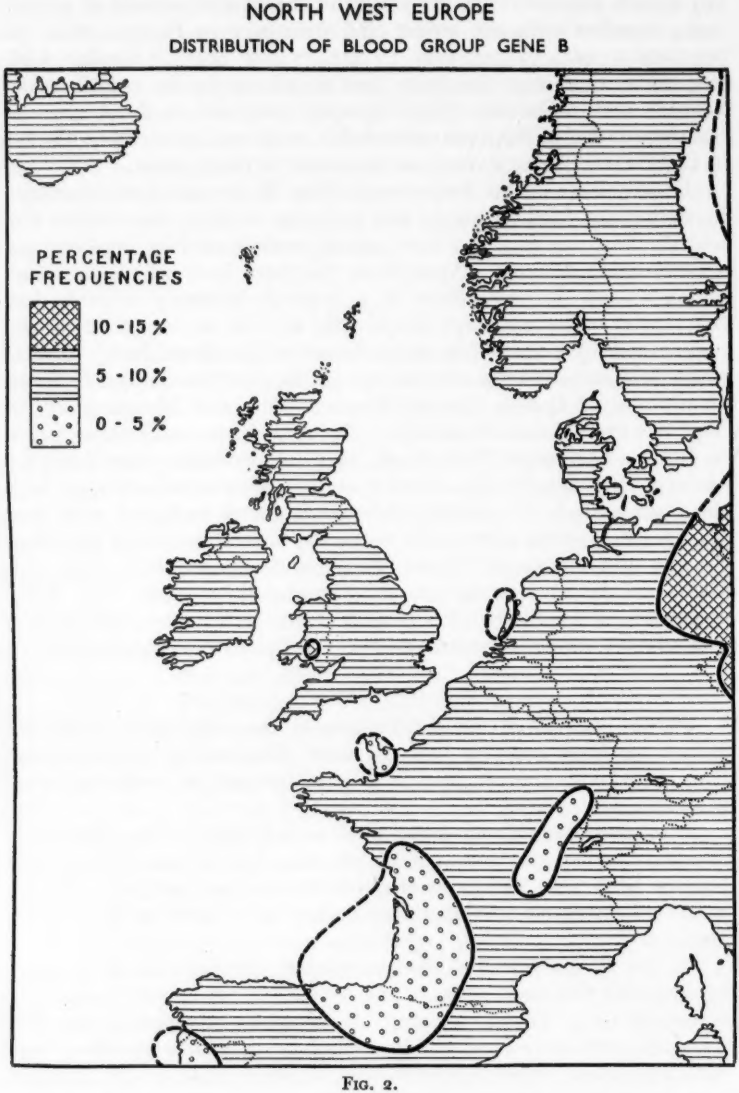


FIG. 2.

When we come to the Mediterranean area, the data are merely suggestive. One is tempted to see a single phenomenon in the

distribution of populations from Galicia along the Mediterranean and on to Iraq and India, all with high *CDe* and low *cde*. It is not

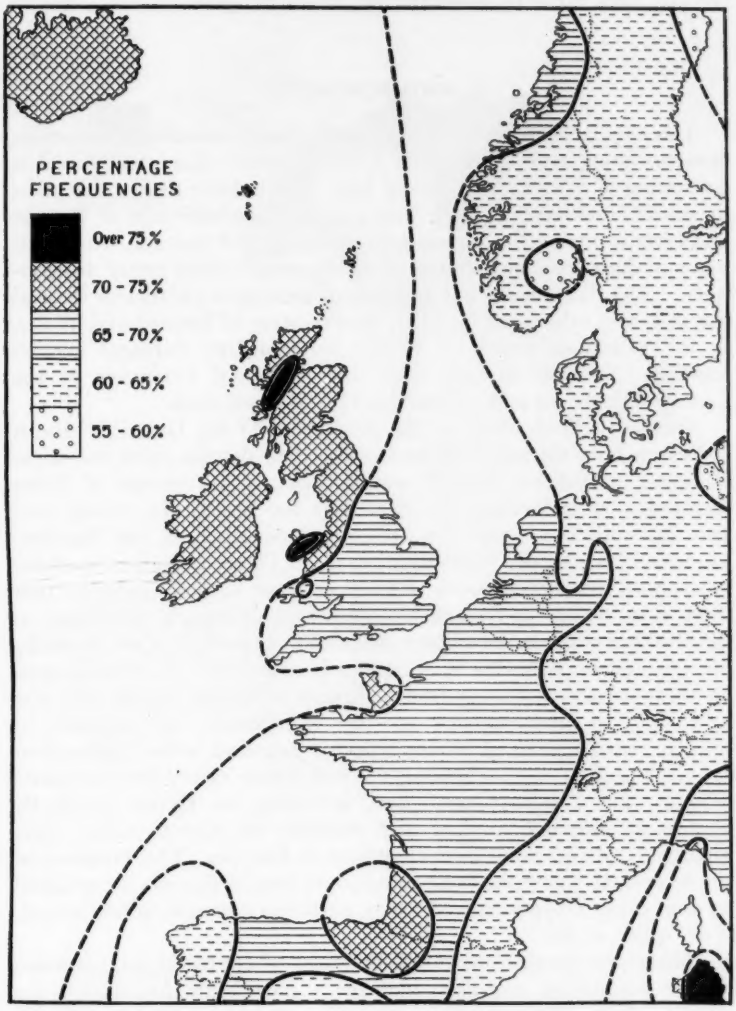


FIG. 3.

clear why the Sardinians with their *ABO* distribution closely resembling that of Iceland, Scotland, Ireland, and parts of Wales, should show

to an extreme degree the very different Mediterranean *Rh* distribution. A series of *Rh* tests on the high group *O* Berbers and on the Western Caucasians with their similar *ABO* frequencies is obviously desirable but not readily attainable. A more detailed study of the distribution of the *ABO* and *Rh* blood groups in the Mediterranean area has recently been made by Mourant (1951).

3. ANTHROPOMETRY

It is beyond the scope of this paper to consider detailed correlations between blood groups and other anthropometric characters, but it is interesting to note them. Fleure and Vallois have drawn attention to resemblances between the late palæolithic inhabitants of Europe and certain small relict populations in islands and mountainous areas. Some of these have been found to show unusual blood group distributions. The Basques are one population showing such ancient skeletal features, and others are found in remote areas of Ireland and Wales, in the Dordogne region of France, in Northern Portugal and in Sardinia. Certain Berbers, and the aboriginal Guanches of the Canary Islands are said to resemble Crô-Magnon man.

Hawkes believes that " a fair proportion of the later inhabitants of Europe have drawn in the network of their descent upon the stocks of upper palæolithic times," whilst Coon, in his survey of living Europeans, puts forward the view that the present-day white race may be reduced to " two least common denominators, the Mediterranean and the Upper Palæolithic group." Fleure and his co-workers, developing the pioneer work of Beddoe, come to the conclusion that there is in Wales " a remarkable persistence of type, a persistence so definite that without the modern Mendelian hypothesis about heredity there would be difficulty in accepting it as a fact." A mountainous country situated at the western periphery of Europe would offer few attractions to later migrants and one is, therefore, not surprised to find in various parts of Wales types of mankind whose distinctions are not transient and accidental, but which date rather from a remote period. The " local types " are, according to Fleure, markedly contrasted with one another and resemble the various racial types identified by anthropologists elsewhere in Europe. The existence of these survivals in considerable numbers in certain districts is explained by him upon a hypothesis of " long persistence or resurgence almost, if not quite, *in situ*."

Three very remote moorlands in Wales where the skeletal features of the population probably indicate a palæolithic foundation are mentioned by Fleure and James. The physical type encountered differs in an important respect from Crô-Magnon Man and seems to point to some other late palæolithic type. Blood group determinations have been carried out upon a sample of the population of each

of the three regions, *viz.* the Black Mountain of Carmarthenshire, the Plynlymon range in Central Wales, and the mountain mass of West Denbighshire termed *Mynydd Hiraethog*. The results of the analysis are shown in table 1.

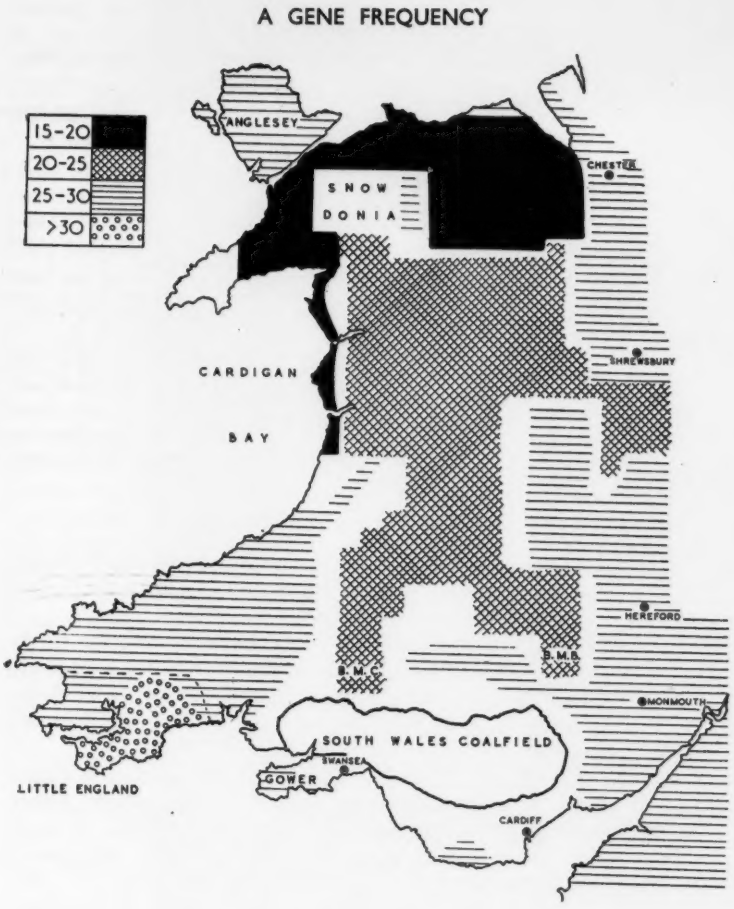


FIG. 4.

It has already been mentioned that the frequency of the *B* gene is higher in the "Celtic" lands than elsewhere in Western Europe, but in these mountain areas it is higher still (fig. 6). The *B* gene in Europe as a whole is mainly associated with the Slavonic speaking peoples and this gene in Western Europe may be derived in part

from the east. The far western rise in *B* is, however, clearly a different and probably a much older phenomenon.

A consideration of some of the details of the distribution of the *B* gene in Wales suggests that it may have been introduced at a very early period indeed in the human settlement of the country.

TABLE 1

Table depicting the wide variation in the frequencies of the O and B genes in Wales

Region	Total	Group frequencies				Expected *AB* per cent.	χ^2 for homogeneity	Gene frequencies		
		O	*A*	*B*	*AB*			*O*	*A*	*B*
Coastal Ardudwy	*550*	55·8	29·8	10·7	3·6	2·4	*2·43*	**75·2**	17·9	6·9
Cardigan Bay (Barmouth-Aberystwyth)	*176*	55·7	29·6	11·4	3·4	2·6	*0·37*	74·9	17·8	**7·3**
Mynydd Hiræthog	*121*	56·2	24·8	16·5	2·5	3·1	*0·11*	**74·7**	**15·0**	**10·3**
Upper Severn System	*1780*	53·1	35·17	8·3	3·4	2·3	*7·02*	73·3	21·2	5·5
Black Mountains (Brecknock and Adjacent Wye Valley)	*713*	52·7	36·0	8·1	3·1	2·3	*1·28*	72·9	21·7	**5·4**
Southern Massif	*1094*	44·6	36·4	14·4	4·6	4·7	*0·01*	66·8	23·2	10·1
Black Mountain (Carmarthenshire)	*161*	39·8	29·8	24·2	6·2	6·9	*0·07*	62·8	20·3	**16·9**
Plynlymon (Southern Side)	*238*	36·5	39·9	18·9	4·6	7·6	*1·72*	**59·6**	**26·6**	13·8

Note.—χ^2 of the (expected-observed) AB has, of course, been calculated from the observed numbers. Owing to the extent of the area, the Upper Severn System displays the heterogeneity which is characteristic of Central Wales as a whole.

The main facts can be summarised as follows :—

(i) The *B* gene reaches its greatest frequency in those regions where the physical anthropologist informs us that the present population contains a palæolithic foundation.

(ii) Even within such areas, however, there is a variation in the frequency of *B*, but the lower value noted on Mynydd Hiraethog agrees with the independent observation of Fleure that " the palæolithic element is less well-marked " in this region.

(iii) The *B* frequency, though lower than in the above-mentioned areas, is, nevertheless, still high in areas where Fleure postulates the existence of a good foundation of early neolithic stock—a stock into which earlier population components had very probably been assimilated. The southern massif extending from North-eastern Carmarthenshire across Northern Brecknock into North-western Radnorshire is one such area.

In the Welsh Basin of the Severn where, according to Fleure, the early neolithic type is manifestly mixed with a tall, fair element, probably coming in across the English border, the frequency of *B* falls to a figure as low as that found in Southern England.

These facts and, in general, the existence of high *B* frequencies among people dwelling on the inhospitable moorlands of Wales—the areas where one would expect the earlier arrivals to have been driven by the later invaders—lead to the suggestion that a high *B* wave entered the country not merely before the moderately high *A* current, but probably even before the very high *O* stream. Relics of the former much wider distribution of the high *B* people are probably to be seen in the moderately high *B* frequencies found elsewhere in Wales as well as in Ireland, Scotland and Brittany.

There is a strong contrast to be observed between the blood groups of the Basques who have the lowest *B* frequency in Western Europe, and this relict population in Wales which has the highest. There is some evidence that both of these populations have been present since palæolithic times and that both were once more widely spread than at present. The suggestion of Chalmers, Ikin and Mourant (1949), and of others, that the ancestors of the Basques once occupied much of Western Europe is thus only a partial key to a situation which may have been as complex as it is to-day.

The close similarity of blood groups between Iceland on the one hand and Scotland, Ireland and parts of Wales on the other, and the difference between Iceland and Norway is a problem of great interest. Donegani, Dungal, Ikin and Mourant (1950) have for various reasons suggested that the original colonists of Iceland may have come mainly from the British Isles, only a relatively small number of a ruling class having come from Norway. Taylor and Fisher (1940) incline to the alternative view that the Icelanders are representatives of the Norwegians of the time of the colonisation, since replaced in Norway by later migrants. Further anthropological investigations at present in progress in Iceland and Western Norway will, it is hoped, shed more light on this question. In support of the former view, however, is the recent discovery that in a certain area of Wales where the available evidence had already pointed to the existence of a Viking colony, the *A* gene frequency of the inhabitants is as high as that of modern Scandinavians. In the comprehensive account of the *ABO* blood group findings in Wales and the Marches which one of us (I.M.W.) hopes to publish shortly, further details concerning this Viking settlement will be given.

That there should exist in most of North Wales, in some mountain regions of South Wales, in Ireland, in Scotland and, to a certain extent, even south of Hadrian's Wall, people whose *ABO* group frequencies are almost identical with those of certain tribes belonging to the North African White Race may, at first sight, seem rather strange. Nevertheless, there is much evidence—anthropological, archæological and linguistic—to suggest that such a finding is more than an accidental coincidence.

The correspondence in the physical anthropology of the two sets

of people was noted by the authors of *Libyan Notes* (1901). They state that the Chawia, for example, are remarkably European in their appearance. Many might pass for Irishmen or Scotsmen. The

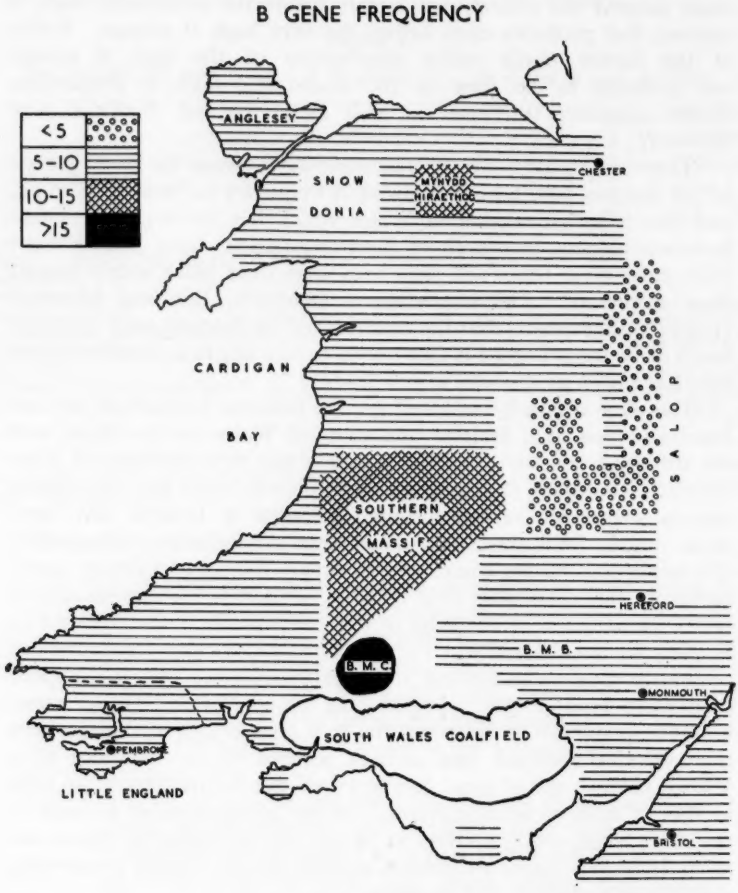


FIG. 5.

children, especially the boys, could if suitably attired, be placed in a class of British pupils and remain almost indistinguishable.

There is abundant archæological evidence linking the Mediterranean basin with Western Britain and Ireland. Cultural relations appear to have been strongest during the neolithic period—the epoch which witnessed the erection of our megalithic monuments. According to Grimes, " the study of the two provinces of North and South Wales

suggests that it was the northern area"—the high *O* area on our map—" which received the western megalithic culture in its greatest vigour."

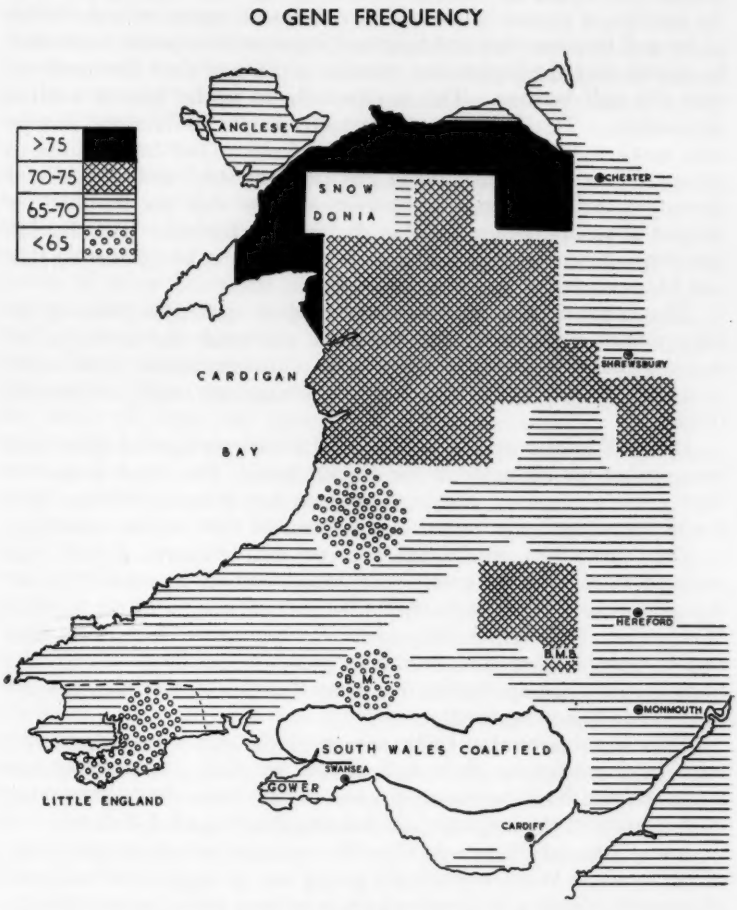


FIG. 6.

4. LANGUAGE

(i) *Philology*

Philologists usually distinguish three different elements in language —sounds, grammar and vocabulary. Many of the older writers held the view that linguistic unity implied racial unity. After the fallacy of this opinion had been exposed, it came to be thought that race

and language were wholly independent of one another. Recently, however, it has become apparent that race plays an important rôle in the determination of the phonetics of a given language. Semantics, on the other hand, are essentially cultural whilst grammar is probably the product of several influences. Even if we disagree as to the width of the gulf between race and language it nevertheless seems reasonable to assume that the further one recedes in point of time the narrower does this gulf become. This is especially so in the case of isolated populations. The Basques are the only people in Western Europe who speak a language that does not belong to the Indo-European group. Thus we see that blood groups, both *ABO* and *Rh*, skeletal characters and language all converge to show that the Basques are distinct from the other peoples of Western Europe. The Basque question is treated, more fully than it can be here, by Chalmers, Ikin and Mourant (1949).

The linguistic peculiarities of the tongues spoken in some of the westernmost outposts of Europe have attracted the attention of many philologists, but nowhere have their researches been more intense than upon the structure of those languages which are termed Celtic.

It is well known that the ancient Celtic tongues have at some time been spoken in all parts of the British Isles. The exact period of their introduction is a matter of dispute, but Pokorny believes that it was coeval with the arrival of the Urnfield Folk, which, according to Fox, was about the eighth century B.C. Though English has replaced them over the greater part of our islands they have, nevertheless, survived in Ireland, Wales, the Isle of Man, and the Scottish Highlands. The Welsh language, together with Armorican and extinct Cornish, form the Brythonic group, whilst Erse, Manx, and Scots Gaelic make up the Goidelic branch. Celtic in its turn belongs to the Indo-European family.

Prior to the arrival of Celtic speech in our islands the inhabitants must have carried on their daily affairs by means of some spoken language, and it, therefore, is of interest to try to ascertain something of the nature of the language which immediately preceded Celtic.

Those who, at the present day, live towards the eastern periphery of Wales where Welsh is gradually giving way to English are witnesses of a process identical with that which must have taken place in Britain over two thousand years ago—the replacement of one language by another. The result is illuminating, for one sees that even in times when a certain degree of education is universal, the replacement is usually far from complete even to the casual hearer, and often decidedly less so to the grammarian. What is largely " a mixed language " ensues in the common parlance though not in the cultured tongue.

In the older language, vocabulary appears to suffer most. When Welsh gives way to English as the individual's mother tongue, relatively few words are handed on from the older to the newer language.

A good illustration is to be found in Old English, which contains but few words derived from its predecessor in England, Brythonic. The present speech of Gascony offers a further example, for though Basque was at one time the language of that region, yet the French dialect of that region to-day contains hardly a word derived from Basque. A search of the Celtic languages for the presence of non-Indo-European words is not, therefore, likely to meet with any great success except in the names of places, of rivers, of mountains, and of valleys, which have a much greater tendency towards permanence. In fact most of the river names in England to-day are Celtic, whilst some may date from an even remoter period. It has recently been shown that in France most of the river names, a great many names of hills, rocks and caverns, and those of many plants and trees are derived from a pre-Celtic language. Furthermore, a comparative study of such names in other European countries has revealed the existence of a common linguistic origin to these terms. The famous Welsh mountain which is similar in shape to a mighty chair and bears the name Cader Idris (Idris' Chair) seems to suggest that Idris was an important personage. When one considers that a line of Berber kings also bore the name of Idriss the coincidence seems noteworthy. It would appear then that long before the invasions of Celtic-speaking tribes much of Europe and also perhaps North Africa was inhabited by a people speaking a common non-Indo-European tongue.

Syntax and idiom, intonation and accent are, however, more stable than vocabulary. In 1621, Dr John Davies, the reviser of the 1588 translation of the Welsh Bible drew attention to the peculiarities of syntax in the Welsh version. He showed that they were alien to Greek and Latin as well as to Hebrew. Much later Rhys mentioned that certain aspects of Welsh syntax as well as some Welsh words were undoubtedly non-Indo-European. Zimmer came to a similar conclusion. Morris Jones was the first to investigate the pre-Aryan syntax in Celtic in a detailed manner. His conclusions have largely been confirmed by Pokorny who has made an exhaustive study of the question with special reference to Irish and has come to the conclusion that " the Celts show in the whole structure of their language a close affinity to the language of the White Mediterranean peoples of North Africa."

A brief summary of some of the more interesting points on syntax put forward by the two last-named workers is given in the Appendix.

Ogham inscriptions are known from Cornwall, Wales, Ireland, Scotland and even as far north as the Shetlands, and among those that are fairly legible there are some of which it can be stated that a knowledge of the Celtic languages has been of little avail in the attempt to translate them. They appear to be written in a language termed by Rhys " Pictish "—a non-Indo-European tongue spoken in the British Isles before the advent of Celtic-speaking people. Latin inscriptions construed in peculiar grammar have also been discovered.

The syntax of these is non-Indo-European and of a type commonly found in agglutinative languages like Basque. Of this peculiar syntax Rhys states: "it occurs in our inscriptions too frequently to be regarded as a slip."

Intonation persists to such a degree that even an educated Welshman can with fair ease be detected by the intonation of his English speech, and the rank and file by idiom and syntax in addition. In parts of Radnorshire where Welsh disappeared as long ago as two centuries, these hallmarks still remain. Even in Hereford town where Welsh has not been spoken since the sixteenth century, the speech intonation that can be heard on a market day is, according to Canon Bannister, still manifestly Welsh. An observer who stood listening to a conversation at such a distance that the words could not quite be caught, he noted, would be convinced that the conversation was proceeding in Welsh and not in English. Traces of a similar intonation may be observed in parts of Gloucestershire and even as far east as Oxfordshire.

The lilting type of intonation found in Ireland and in much of Wales and the Marches, which is so alien to standard English, may also be observed, according to Byrne, in some of the North African languages. This finding has not, so far, been confirmed.

It is but a short step from language and its intonation to oratory, and one of the distinguishing features of pulpit oratory in Wales is the *hwyl*. To drive his message home more effectively, the preacher may depart from normal speech in sermon delivery and proceed in the form of a chant. The greater the response from the congregation —in Nonconformist Wales this can, in large measure, be judged by the frequency of the exclamations of approbation—the more fully developed is this chant liable to become. All preachers do not follow the identical musical score, but the underlying pattern is the same in all cases.

T. Gwynn Jones was the first to draw attention to the similarity of the Welsh *hwyl* to the chant of the African Muezzin. More recently, the B.B.C. produced a feature programme upon the subject. Sound recordings of some Welsh preachers on the one hand, and the white North African's call to prayer on the other, were broadcast. The two were strikingly similar. But the record of one Welsh preacher in a fervent *hwyl* was apparently so very nearly identical with the Muezzin's chant that it was possible to interplay the records and, unless one made a special effort to grasp the words, it was impossible to discover where the North African was cut out and his place taken by the Welsh preacher.

(ii) *Phonetics*

Linguistic problems may, however, be approached from quite a different angle from the above. Darlington has given reasons for supposing that the sounds of a language as distinct from its etymology

and grammar are largely in the long run determined by genetical characters of the vocal organs. In particular, he has shown that there is a close correlation in Europe between a high frequency of the blood group *O* gene and the presence of a *th* sound in the language. It would be interesting to see this supported by anatomical data.

We have seen that the Basques are probably a particularly pure relict race. They have a high *O* frequency and a strongly developed *th* sound (or rather a *tth* sound usually represented in writing by the letter *z*). If, therefore, only one stock has happened to carry the combination of many *O* genes and the ability to pronounce *th*, we should expect to find a large proto-Basque element in all the high group *O th* speakers, and hence a high correlation between the typically Basque *d* gene, the *O* gene and the *th*-speaking character. Alternatively, the Basques may represent a fusion of an *O*, *th*-speaking stock with a *d* stock.

We have noted that in several regions in the Old World showing a similarity in blood group frequencies of their inhabitants, the languages spoken also display a number of phonetic peculiarities in common. A detailed examination of this most interesting problem is, however, beyond the scope of the present paper but we may mention two examples, the *ll* in Welsh and Icelandic, and the mutation of consonants in Welsh and Irish and also in the Sassaric dialect of Sardinia.

5. ANCIENT RECORDS

Historians and early chroniclers have added to our knowledge of ancient Britain. That there were distributed in the far west of Europe —in an area apparently similar to that in which people of very high *O* frequencies are found to-day—national communities completely distinct from the Celts, was known to Herodotus. " The Celts," he writes, " are outside the Pillars of Hercules and they border on the Kynesii who dwell the furthest away towards the west of the inhabitants of Europe." He repeats the statement in his fourth book where he states that the Celts are the furthest away towards the setting of the sun, " with the exception of the Kynetes " (*sic*).

The duality of the population in Southern Britain at the time of the Romans is considered by Huxley to be " one of the few fixed points in British ethnology." Strabo informs us that the prisoners taken in the south-eastern part of Britain were six inches taller than the tallest men in Rome. Their colouring was blond. In the interior on the other hand, there dwelt according to Caesar, a vastly different people who regarded themselves as the autocthonous children of the soil. The swarthy visages and twisted locks of the South Welsh tribe of Silures, states Tacitus, pointed to their Iberian origin. (The territory occupied by the Silures includes the high *O* area of the Brecknock Black Mountains and the adjacent Wye Valley.) The very name " Silures " has defied an explanation from Celtic sources.

The customs of these aboriginal islanders contrasted materially with those of the later comers who differed in this respect but little from the Gauls. The institution of marriage, as practised by the Romans, was unknown to the former and, as a consequence, every relationship was counted through the mother. Schrader states that the primeval Aryan family was, on the other hand, patrilineal. In referring to the matrilineality of the early inhabitants of Britain Zimmer writes : " Auf einen Piktenherrscher und seine Brüder folgt nicht etwa der Sohn des ältesten, sondern der Sohn der Schwester." Amidst the Berbers and in other parts of the East, the same state of affairs exists to this day. Hanoteau states of the former : " Quand le roi meurt ou est déposé, ce qui arrive assez souvent, ce n'est pas son fils qui est appelé à lui succéder, mais bien le fils de sa soeur."

At what period the non-Indo-European tongue of the British Isles became extinct is not certain, but it appears not to have been long dead in Munster when Cormac wrote his Glossary in the ninth century A.D. He refers to it as the " iron language "—a name which it received on account of the difficulty of seeing through it, and owing to the darkness and compactness of its texture.

Adamnan wrote the *Life of Columba* towards the close of the seventh century and his work is available to us in a manuscript from the eighth. He relates how Columba, after having come over from the north of Ireland to Britain, went on a mission to Brude, King of the Picts, whose stronghold lay near the mouth of the river Ness. To the king and his court he had no difficulty in making himself understood, but on journeying inland one reads of his preaching to the peasants with the aid of an interpreter. At a later date, on the island of Skye, two young men brought their father to him to be baptised. Here again, he was forced to enlist the services of an interpreter although the convert bore the Celtic name of Artbrannan.

That these were far more than dialect differences which could easily be mastered is pointed out by Rhys. Furthermore, intra-Gaelic dialect differences were, at most, of relatively little importance at this early stage. The conclusion one can draw is that the language was pre-Celtic.

Bede lists the people of Britain in whose languages Christianity had in his day to be taught as being the Angles, the Brythons, the Scotti, the Picts and the Latins. The language of the Picts was, therefore, distinct from that of the Scots—Gaelic, and from that of the Brythons. Rhys states that the significance of Bede's words is sometimes explained away by supposing Pictish to have been a Celtic dialect lying somewhere between Brythonic and Goidelic. There is, however, little reason to suppose that this was Bede's view ; for in the case of English he was content to let the language of the Angles stand for all dialects without mentioning, for instance, that of the Saxons.

6. DOMESTIC ANIMALS

Much information on human migrations may be gleaned from a historical study of the genetics of those domestic animals to which early man was perforce bound. Adametz was the first to study this question in a scientific manner. According to him, the primitive North African long-horn cattle and the greyhound are native to the Hamitic north of the African continent. The long-horns have not only spread throughout North-eastern and South Africa, but across Spain (Andalusian cattle) and as far as Britain where the Black Cattle of Wales and the Reds of Devon are the undoubted descendants of the early North African stock, whilst the Herefords and the Highland cattle are very probably so. The Scottish deerhounds and the Irish wolfhounds are, according to Hilzheimer, crosses between the Hamitic greyhound and Scottish and Irish wolves respectively. It may become possible in the near future to test some of these relationships by means of the blood groups of the animals concerned.

7. CONCLUSION

In view of the evidence presented there appear to us reasonable grounds for the belief that, prior to the advent of Celtic-speaking immigrants, the British Isles were inhabited by a people whose domain had at one time extended over a considerable part of Europe and North Africa but who under ever increasing pressure from the east had been driven from their homelands. Some, no doubt, found refuge in the more isolated mountain regions, but the remainder were gradually driven westwards and finally came to occupy a limited area near the Atlantic seaboard of Europe. That the descendants of these people have, to this day, remained largely unmolested in those parts of our islands furthest away from the ancient land connection with the continent of Europe seems demonstrated in the distribution of their *ABO* genes, which singles them out from the other people of Northern Europe, excepting the Icelanders, but links them with the Berbers and other communities living on the Mediterranean shore and in the Middle East.

The pattern upon which the early peregrinations of *Homo sapiens* was woven is gradually becoming clearer, but the main lesson to be drawn is the need of correlation of the steadily accumulating blood group data with all the other sources of information—physical, cultural, linguistic and documentary.

8. SUMMARY

1. In Europe three main zones of *ABO* blood group distribution can be distinguished, one of relatively high *B* in the east, one of high *A* in the centre and west and one of high *O* in the extreme west, with *B* slightly but significantly more frequent than in the central zone.

The *Rh* groups are fairly evenly distributed in Northern and Central Europe with about 16 per cent. of *Rh*-negatives. The frequency of *Rh*-negatives is about 30 per cent. in the Basques and varies from 5-16 per cent. in Southern Europe.

2. Wales displays significant local variations in the frequencies of the *O*, *A* and *B* genes. On the remoter moorlands where physical anthropologists postulate the existence of very early human stocks, *B* gene frequencies exceeding 10 per cent. are common, and in the Black Mountain of Carmarthenshire even exceed 16 per cent. In the Marches they fall below 5 per cent.

3. Apart from one or two mountain regions in South Wales, very high *O* gene frequencies of 70-75 per cent. similar to those found in Scotland and Ireland are confined to the north of the principality—a fact which supports the obvious view that a less accessible North Wales has not been subjected to the human migrations which have affected the southern half of the country.

4. It appears that a high *B* wave entered Wales not merely before the moderately high *A* current, but probably even before the very high *O* stream. It has formerly been supposed that populations with high *B* frequencies were among the latest to enter Europe and were confined to the east.

5. *O* frequencies similar to those in North Wales, Scotland and Ireland are also to be found among the Berbers in North Africa and elsewhere in the Mediterranean region. That the early inhabitants of Western Britain had strong White North African affinities is suggested by the pre-Aryan syntax of Celtic. Philologists have pointed out that " the Celts show in the whole structure of their language a close affinity to the language of the White Mediterranean peoples of North Africa."

9. APPENDIX

Pre-Aryan survivals in Celtic syntax

It is contended by Morris Jones and Pokorny that if the pre-Celtic inhabitants of Britain are related to the Berbers of North Africa it is not unnatural to expect that their language should also have belonged to the Hamitic family—a family which includes the Berber languages and ancient Egyptian.

It is known that Egyptian preserves a form of Hamitic speech and there is reason to believe that it approaches the ancient type of Hamitic language much more closely than any of the modern Berber dialects. Consequently, it is Egyptian that one expects to bear the greater resemblance to the pre-Celtic speech of our islands.

(i) *Sentence connections*

One of the most characteristic features of the neo-Celtic languages is the tendency to break speech into a series of direct clause statements. Instead of there being a single sentence of some length comprising one or more subordinate clauses, as is seen in Latin, English, German, etc., one finds a sequence of short simple sentences. As it is recognised that subordination of sentences is commoner in written than in spoken language, the relative rarity of subordination in written Irish is all the more remarkable and suggests that the agglutinative character of the language

is fundamental. The Indo-European relative sentence is not mirrored in the neo-Celtic languages except partially. A basic difference exists between a relative sentence in which the relative is the subject or object and that in which it is in an oblique case. Personal pronouns such as " whose," " of whom," " of which," corresponding to the Latin " cuius," " quorum," " quarum," are absent. For example " I gave it to the man who delivered the bread " can be rendered into Irish or Welsh on the same relative Indo-European pattern as in English. But " I gave it to the man whose house lies at the top of the hill " cannot. It would seem as though a statement such as the latter was divided up in ancient times somewhat as follows : " I gave it to the man. The man's house lies at the top of the hill." A gradual infiltration of Indo-European influence in this particular can be noted as one advances through the centuries from Old Irish and Old Welsh to the mediæval forms and on to modern speech.

It is well known that the asyndeton—a figure of speech which omits connectives, as " I came, I saw, I conquered "—plays an important rôle in Egyptian. There is an aversion from the use of subordinate sentences in both Egyptian and Berber. Thus, Irish and Welsh in the essentials of their speech construction point unmistakably towards North Africa.

(ii) *Word order*

The relation of words in the Indo-European sentence being sufficiently demonstrated by inflexions, the order of words may vary, but it is the verb which usually comes *last*. In Irish and Welsh, on the other hand, the verb normally comes *first* : thus, in Welsh, *Cododd Arthur y garreg*, " Arthur picked up the stone " ; in mediæval Irish, *Aliss Patricc Dubthach*, " Patrick requested Dubthach." O'Donovan tells us in his *Irish Grammar* that " In the natural order of an Irish sentence the verb comes first, the nominative with its dependents next after it, and next the object of the verb." Ranouf in his *Egyptian Grammar* writes : " The order of the words in an ancient Egyptian sentence is constant. When the verb is expressed, it precedes the subject. If both the nearer and the remote objects of a verb are nouns, the former is placed after the subject and the latter comes last."

There is a special form of sentence in Welsh in which a noun as subject comes first. This order is used to lay particular stress upon the subject ; thus *Duw a farn . . .* ; for (*Yr*) *Duw a farn . . .* ; " (it is) God who judges . . ." Similar syntax may be heard in the English spoken on the streets of Cardiff on the occasion of an international rugby match ; thus, " From the Rhondda I am " for " (It is) from the Rhondda I am." In Egyptian, according to Ranouf, " a noun at the beginning of a sentence implies the ellipsis of the verb ' to be '."

In both Irish and Welsh an adjective, or noun in the genitive case, is placed after the noun which it qualifies ; in Welsh *gwr mawr*, Irish, *fear mór* " a great man." The same order exists in Egyptian. Bergaigne has pointed out that the qualifying word, whether adjective or genitive, came *before* the word in the primitive Indo-European sentence. There is, therefore, in Irish and Welsh a divergence from the primitive Aryan order in the sentence and its replacement by the same order as is found in Hamitic—a divergence which is also noticeable in some of the Romance languages which, as is known, display in the syntactical field certain pre-Aryan traits.

(iii) *Inflected prepositions*

In the neo-Celtic languages when the object of a preposition happens to be a personal pronoun, it becomes attached to the preposition in a manner identical with an inflexion. The fusion is complete for the appendage is not even hyphenated ; thus in Welsh " us " is *ni*, but " for us " is not *er ni*, but *erom*. In Egyptian the endings forming personal verbs are also affixed to prepositions.

The preposition *yn* which cannot be adequately rendered into English by a single word is so remarkably like the Egyptian *em* in its syntactical usages that

mention must be made thereof. In common with other prepositions, both the Welsh *yn* and the Egyptian *em* take pronominal suffixes, as explained above ; both are used in the sense of " in " before a place name ; both are used to introduce the complement after verbs of being and becoming. Their chief characteristic is their usage as introduction in every kind of complement. *Yn* like the Egyptian *em* is used after the verb " to be " to introduce not only an attributive substantive but also an attributive adjective.

(iv) *Periphrastic conjugations*

In Egyptian one of the commonest forms of periphrastic conjugation is as follows :—(*a*) verb " to be " followed by a noun or having a personal suffix joined to it ; (*b*) a preposition ; (*c*) verbal noun. Although Irish and Welsh retain many Indo-European tenses, the type of conjugation mentioned above is, nevertheless, extremely common. In Egyptian, the preposition *em*, " in," *er* " to " or " for," *her* " above " or " upon " are used to indicate the present, future and perfect tenses respectively. In Welsh the corresponding prepositions are *yn* " in," *wedi* " after," while *am* " for," presumably an old future, is now only used optatively. For example : *Maent wedi cael bwyd* (literally) " They are after having food." This does not mean " they are in quest of food," but the exact opposite " they have had food." Syntax of this kind may also be heard in Irish-English. Yet one must remember that a somewhat similar type of construction is to be found in English, though its source appears unknown, *e.g.* he is a-coming ; a-hunting we will go. Probably it has filtered through to English.

The syntactical features mentioned above with particular reference to Egyptian are exhibited fairly consistently by all the present-day Berber dialects. The Semitic languages which are related to Hamitic also display many of these features, but they agree less closely in syntax with the Celtic languages.

Acknowledgments.—We are greatly indebted to Dr C. D. Darlington of the John Innes Institution and Dr J. R. Fraser Roberts of the London School of Hygiene and Tropical Medicine for offering us a wealth of helpful criticism and advice on several aspects of the work. We thank Dr R. J. Drummond, Medical Director of the National Blood Transfusion Service, Wales, who placed at our disposal the record cards of the area under his jurisdiction and consistently assisted the investigation by arranging blood donation clinics in the areas of greatest anthropological interest. We also thank Dr D. E. Lehane of Liverpool for allowing us to analyse the records of the groupings relating to Anglesea and Cheshire as well as those of Caernarvonshire, Denbighshire and Flintshire which had become available since Dr Fraser Roberts' survey of 1942. We are grateful to Dr W. Weiner of Birmingham who gave us permission to inspect the records of Herefordshire, Shropshire and Worcestershire and to Major R. Deedes, Honorary Organiser of the Transfusion Service in Shropshire, who materially assisted us with the sorting of the cards in his area. We also wish to thank Dr H. J. R. Kirkpatrick, Medical Director of the North of Scotland Blood Transfusion Service, Inverness, who has allowed us to use the results of his unpublished analysis of the distribution of the blood groups in northern Scotland.

10. REFERENCES

(a) *Blood groups*

AGOSTI ROMERO, L., IKIN, ELIZABETH W., AND MOURANT, A. E. 1950. Les groupes sanguins ABO, MNS et Rh des Galiciens. *Rev. Hemat.*, *5*, 325-328.

BOYD, W. C. 1939. Blood groups. *Tabul. Biol.*, *17*, pt. 2.

CHALMERS, J. N. M., IKIN, ELIZABETH W., AND MOURANT, A. E. 1949. The ABO, MN and Rh blood groups of the Basque people. *Amer. J. Phys. Anthrop.*, *n.s.*, *7*, 529-544.

CHOWN, B., PETERSON, R. F., LEWIS, MARION, AND HALL, ANN. 1949. On the ABO gene and Rh chromosome distribution in the white population of Manitoba, Canada. *J. Res., E., 27,* 214-225.

DE HOYOS SAINZ, L. 1947. Distribucion de los grupos sanguineos en España. *Instituto "Juan Sebastian Elcano," Consejo Superior de Investigaciones Cientificas, Madrid.*

DOBSON, A. M., AND IKIN, E. W. 1946. The ABO groups in the United Kingdom, etc. *J. Path. and Bact., 58,* 221-227.

DONEGANI, JOYCE, DUNGAL, N., IKIN, ELIZABETH W., AND MOURANT, A. E. 1950. The blood groups of the Icelanders. *Ann. Eugen., 15,* 147-152.

ETCHEVERRY, M. A. 1945. El factor Rhesus, su genética e importancia clinica. *Día. Méd., 17,* 1237-1251.

HART, E. W. 1944. An analysis of the blood group composition of a population in Northern Ireland. *Ann. Eugen., 12,* 89-101.

KAYSSI, A. 1949. The Rh blood groups of the population of Baghdad. *Amer. J. Phys. Anthrop., n.s., 7,* 549-551.

MORGANTI, G., PANELLA, I., AND CRESSERI, A. 1950. Distribution des types Rh dans la population italienne. *Rev. Hémat., 5,* 329-333.

MOULINIER, J. 1949. The Rh factor in south-western France. *Amer. J. Phys. Anthrop., n.s., 7,* 545-548.

MOURANT, A. E. 1951. The blood groups of the peoples of the Mediterranean area. *Cold Spring Harbor Symposia on Quantitative Biology, 15* (in the press).

RACE, R. R., LAWLER, SYLVIA, D., BERTINSHAW, DOREEN; GRIFOLS LUCAS, J. A., GRIFOLS LUCAS, V., IBARZ ROQUETA, M., AND OPPENHEIMER, W. 1949. Los genotipos Rh de 223 dadores de sangre españoles. *Medicina Clinica, Barcelona, 13,* 325-326.

RACE, R. R., AND SANGER, RUTH. 1950. *Blood Groups in Man.* Oxford: Blackwell Scientific Publications.

RACE, R. R., SANGER, RUTH, AND SELWYN, J. G. 1950. A probable deletion in a human Rh chromosome. *Nature, 166,* 520.

ROBERTS, J. A. FRASER. 1942. Blood group frequencies in North Wales. *Ann. Eugen. Lond., 11,* 3, 260-271.

ROBERTS, J. A. FRASER. 1948. The frequencies of the ABO blood-groups in South-Western England. *Ann. Eugen. Lond., 14,* 2, 109-116.

SACHS, H. 1940. Some aspects of the problem of blood grouping and the distribution of blood groups in the Irish population. *Iris J. Med. Science,* April 1940.

TAYLOR, G. L., AND FISHER, R. A. 1940. Scandinavian influence in Scottish ethnology. *Nature, 145,* 590.

VALLOIS, H. V. 1944. La répartition anthropologique des groupes sanguins en France et plus spécialement dans le Sud-Ouest. *Bull. Mém. Anthrop.,* Paris, ser. 9, *5,* 53-80.

VALLOIS, H. V. 1949. La répartition des groupes sanguins en France: l'ouest Armorico-Vendéen. *Archiv der Julius Klaus-Stiftung für Vererbungsforschung, Sozialanthropologie und Rassenhygiene, 24.*

(b) *Anthropometry*

COON, C. S. 1948. *The Races of Europe.* New York.

FLEURE, H. J., AND WHITEHOUSE, W. E. 1916. Early distribution and valleyward movement of population in South Britain. *Arch. Camb., 16,* 101-140.

FLEURE, H. J., AND JAMES, T. C. 1916. Geographical distribution of anthropological types in Wales. *J.R.A.I., 46,* 35-172.

FLEURE, H. J. 1920. Some early neanthropic types in Europe and their modern representatives. *J.R.A.I., 50,* 12-40.

VALLOIS, H. V. 1943. *Anthropologie de la population française.* Paris.

(c) *Language*

BEDE. 1912. *Historical Works*, ed. C. Plummer. London.
BYRNE, J. 1885. *General Principles of the Structure of Language*. London.
DARLINGTON, C. D. 1947. The genetic component of language. *Heredity*, *1*, 3, 269-286.
DAUZAT, A. 1940. *L'Europe linguistique*. Paris.
DAUZAT, A. 1946. *La Toponymie française*. Paris.
DAUZAT, A. 1947. *Les noms de lieux*. Paris.
DAVIES, J. 1621. *Antiquae linguae Britannicae*. Londini.
HANOTEAU, A. 1860. *Grammaire tamachek'*. Paris.
JONES, J. MORRIS. 1900. The pre-aryan syntax in insular Celtic. Appendix B of *The Welsh People* by J. Rhys and D. Brynmor Jones, London.
LEWIS, H., AND PEDERSEN, H. 1937. *A Concise Comparative Celtic Grammar*. Göttingen.
MEILLET, A. 1918. *Les langues dans l'Europe nouvelle*. Paris.
POKORNY, J. 1927-30. Das nicht-indogermanische Substrat im Irischen. *Zeitsch. f. Celt. Philologie*, *16*, 95-144, 231-266, 363-394 ; *17*, 373-388 ; *18*, 233-248.
POKORNY, J. 1933. Origin of the Celts. *Nature*, *132*, 648.
RHYS, J. 1879. *Lectures on Welsh Philology*. London.
TAYLOR, I. 1865. *Words and Places*. London.
ZIMMER, H. 1913. Aus dem Nachlass Heinrich Zimmer's, ed. Kuno Meyer. *Zeitsch. f. Celt. Philologie*, *9*, 87-120.

(d) *General*

ADAMETZ, L. 1920. *Herkunft und Wanderungen der Hamiten*. Vienna.
ADAMNAN. 1920. *Life of St Columba*. (Reeves ed.) Oxford.
BANNISTER, A. T. Personal communication.
B.B.C. Welsh Home Service feature programme *Yr huyl gymreig*. Produced by A. Talfan Davies, 2nd December 1945.
CAESAR. *De Bello Gallico*, *5*, 14.
CORMAC. 1912. *An Old Irish Glossary*, ed. Kuno Meyer. Halle.
DANIEL, G. 1950. *A Hundred Years of Archæology*.
FOX, C. 1947. *The Personality of Britain*. Cardiff.
GRIMES, W. F. 1936. The megalithic monuments of Wales. National Museum of Wales, reprint from *Proceedings of the Prehistoric Society*, 1936.
HAWKES, C. 1940. *The Prehistoric Foundations of Europe*. London.
HERODOTUS. *2*, *4*.
HILZHEIMER, M. 1926. *Natürliche Rassengeschichte der Haustiere*. Berlin.
HUXLEY, T. H. 1890. *Critiques and Addresses*. London.
JONES, T. GWYN. 1912. *Môr canoldir a'r Aifft*. Caernarvon.
RANDALL-MACIVER, D., AND WILKIN, A. 1901. *Libyan Notes*. London.
RHYS, J. 1904. *Celtic Britain*. London.
SCHRADER, O. 1890. *Sprachvergleichung und Urgeschichte*. Iena.
STRABO. *4*, 4.
TACITUS. *Agricola*, *11*.
ZIMMER, H. 1894. Das Mutterrecht der Pikten. *Zeitsch. f. Rechtsgeschichte*, *15*, 209-240.

BOLTING, A VEGETATIVE VARIATION IN THE POTATO

W. R. STANTON

John Innes Horticultural Institution, Bayfordbury, Hertford

Received 20.vii.51

CONTENTS

1. Introduction 37
2. Effect of Environment 39
3. Evidence from Grafting 45
4. Breeding Results 48
5. Discussion 50
6. Summary 52
7. References 52

1. INTRODUCTION

Potato varieties are conveniently classified according to the time the tubers mature and the crop is ready for harvesting as early (E_1), second early (E_2) maincrop (E_3) and late varieties (E_4). In approximately 80 per cent. of the early varieties (E_1, E_2) but in only 20 per cent. of the main crop (E_3) and apparently never in the later varieties abnormal plants may be found. These differ from the type in several respects. They have taller haulms, flower abundantly, and continue to grow when the normal crop is mature. The tubers they produce are coarser, deeper-eyed and are borne on longer stolons than the normal plants, from which the new types arise as vegetative sports. They vary greatly with environmental conditions and lead to much speculation as to the cause and origin of these plants.

This aberrant condition in the potato commonly called " Bolting " has long been recognised as a trouble of the growers of potato tubers for " seed," as they have constantly to rogue their stocks. A search for the cause of the aberration started about 1920 and from 1927 until quite recently the condition has been classified with the virus diseases (Folsom, 1927 ; McIntosh, 1931 ; Murphy, 1932 ; Hansen, 1936). Prior to 1927 it had been classified as a degenerative change of unknown origin (Anderson, 1925 ; Salaman, 1926). In the United States an aberration called " Giant Hill " has been shown to be identical with bolting by Murphy and McKay (1932).

Photoperiodic reaction

The study of the photoperiodic reaction of the South American potatoes began with the work of Hackbarth (1935) and has been followed by the extensive work on the Empire Potato Collection made by Driver and Hawkes (1943). These studies showed a similarity

between the normal-bolter change in our cultivated varieties and the change in appearance of short-day adapted *Solanum andigenum* clones when grown under long-day conditions. That the bolter behaves as a short-day adapted potato has been subsequently demonstrated by Hawkes (1947).

Occurrence

How bolting arises and how it is inherited are still uncertain. In vegetative propagation the condition can apparently exist for many years (Davidson, 1937) and, according to Yarwood (1946), it is possible to retain the identity of a number of " Giant Hill " strains from year to year. However such differences have not been precisely recorded in this country, although growers commonly speak of semi- and super-bolter conditions. The frequency with which new bolters may arise somatically may be as high as 1 per cent., so Dr Black tells me, but accurate information is still lacking on this point.

Preliminary studies on the inheritance of the condition have yielded unsatisfactory results (Carson and Howard, 1944 ; Revell, 1947), the difficulty in scoring being due to the wide variation in the appearance of the bolter itself and also the progeny.

Degrees of bolting are sometimes mentioned (Davidson, 1937 ; Scottish Department Agriculture, 1944). They have been called semi-bolter, full bolter and super-bolter. Semi-bolters are often so little differentiated from the normal that reports about them are unreliable. Super-bolters are apparently rare ; occurring with a frequency of the order of one-hundredth of that of the equivalent bolters. The three conditions will be called in this paper semi-bolter B−, full bolter B and super-bolter B+. Experiments have been conducted only on the bolter (B).

The relation, in terms of lateness of maturity, of the various types of bolter is shown in fig. 1. Examples are given of the behaviour of varieties from each of the groups E_1, E_2 and E_3.

The differences between the normal and bolter (B) conditions may be summarised as follows and the " time of planting experiment " subsequently described has shown the relative value of some of the following characters for general application in the scoring of field plots.

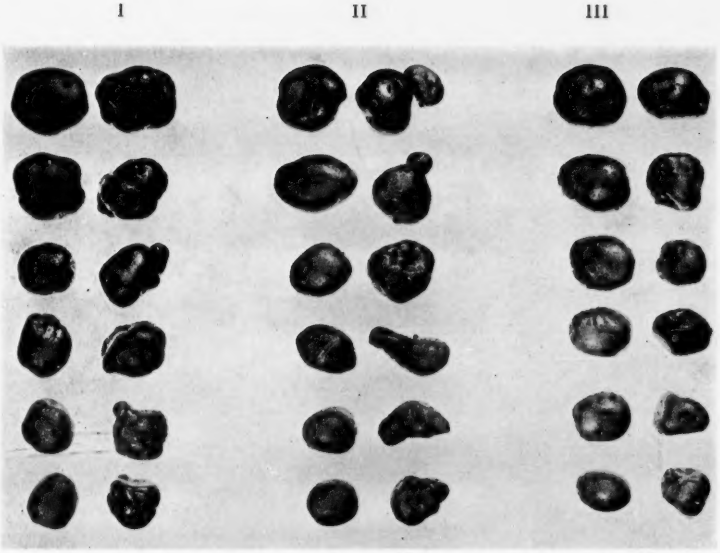
Flowering

Bolters flower abundantly, more initials being produced and more flower buds reaching maturity. Flowers appear earlier and more often and bear fruit on normally unfruitful varieties.

Haulm growth

The haulm is taller and more robust. The leaflets are thicker and, with high nitrogen nutrition, puckered due to interveinal growth.

Plate I



Selected tubers of three varieties from adjacent plots: I Edzell Blue, II Gladstone, III Doone Star, showing the difference between Normal N. (left) and Bolter B. (right).

Tuber formation

This is delayed so that an early variety (E_1 or E_2) is in effect converted into maincrop or late variety (E_3 or E_4). The tubers, relatively numerous, are borne on longer stolons and may show secondary growth.

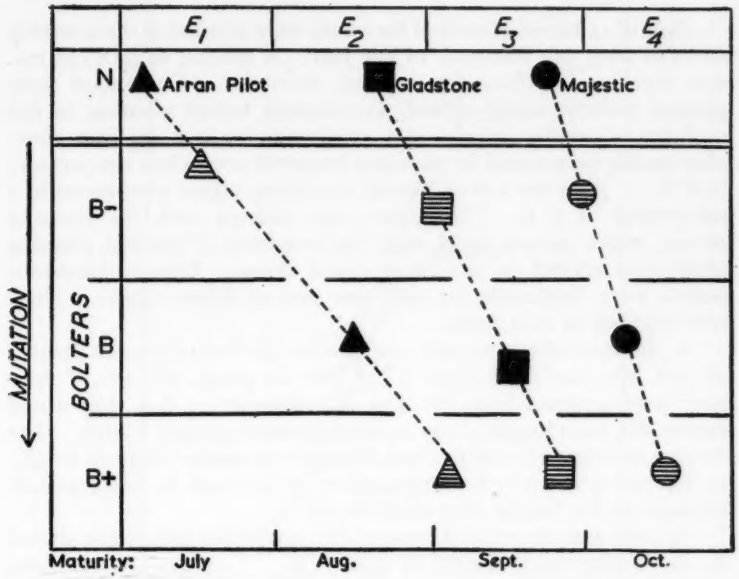


FIG. 1.—Diagram showing the change of maturity in three varieties accompanying the change Normal-Bolter.

E_1, E_2, E_3, E_4, Classification of maturity. N = Normal. B− = semi-bolter; B = bolter; B+ = super-bolter. (For details of the differences between these three types see text.) Note that the "maturity-shift," towards later maturity, changes according to the time of maturing of the original variety. The greatest maturity-shift occurs with early varieties.

The location of points on the bars B− and B+ are tentative. The difference in the time of maturing between the N and B− is hard to detect.

Tuber shape

Tubers of bolters are rougher skinned, on the average less regular in shape, and with deeper eyes. In kidney shaped varieties the tubers may become spindle shaped (see plate I).

2. EFFECT OF ENVIRONMENT

To study the variation of the bolter (B) condition, a series of plots containing normals and bolters was laid out in the spring and summer

of 1948. The following varieties, kindly supplied by Dr W. Black of the Scottish Plant Breeding Station, were used :—

Arran Pilot (E_1)	Normal and Bolter (B)
British Queen (E_2)	Normal
Gladstone (E_2)	Normal and Bolter (B)

Plots of 15 tubers of each of the above were planted at three-weekly intervals from 9th February to 5th July. A spacing of 90 × 135 cm. was necessary to allow for detailed excavation. The tubers were planted without being chitted, *i.e.* exposed before planting to full sunlight on benches or trays in a greenhouse for four weeks or more, after having been stored in trays in a basement with a low temperature (5-8° C.). After the end of March, remaining tubers were stored in a refrigerator at 5° C. The tubers were planted with the rose-end 10 cm. below ground level with the exception of the last planting which was started in pots in a closed frame. Enough Gladstone bolters were obtainable for only one plot of fifteen tubers. These were planted on 12th April.

A photoperiodic treatment was given to the two plot series planted on 3rd May and 15th June. The first six plants of each of these plots were screened from the time of emergence so that they would receive the same length of day as those planted on 22nd March. The diurnal duration of covering was changed at weekly intervals to give an increasing day-length corresponding to the week by week natural increases in day length after 22nd March.

As previously mentioned, several characters of a variety are altered by the change from normal to bolter (B). Two characters, haulm height and stolon length were recorded in detail, and as the full normal-bolter comparison was possible only for the variety Arran Pilot (E_1), observations on this comparison are confined to this variety.

Haulm height was measured at 10-day intervals from the time of mean emergence to the maturity of the normal plants of a particular planting time. Except for the last series (5th July) which were lifted immature, lifting of normals and bolters took place at the time of maturity of the normals (see figs. 2*a* and 2*b*, and table 1).

The form of the growth curve is shown by the graph (fig. 2*a*) of the mean haulm heights of the different plots of the 3rd May planting from emergence to maturity of the normal plants. It will be seen that the slope of the curves for normal and bolters is the same. The characteristic difference between them being the greater length arising from longer duration of growth of the bolter curve as compared with the normal.

This difference is shown in fig. 2*b* for the various planting times. This graph gives the mean maximum height of the normals, with

their different planting dates, the mean maximum height of the corresponding bolter plots at the same time, and the difference between them.

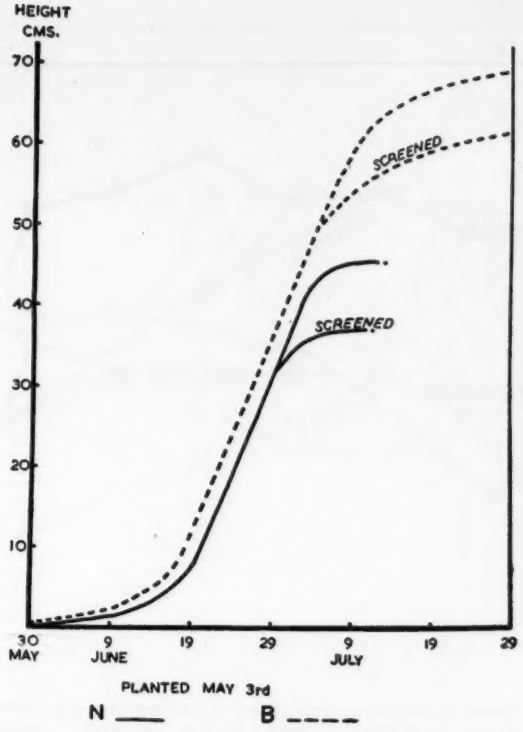


FIG. 2a.—Increase in height of plants from mean ground level (plot mean) measured at 10-day intervals. The height taken was to the apex of the tallest haulm.

The plants were lifted by excavating a trench along each side of the row and teasing out the soil between the stolons. All stolons bearing tubers of over 10 gms. were measured and fig. 3 shows the variation in the mean stolon length according to the time of planting. Mean stolon length is increased with lateness of planting, but the stolons of bolters are longer than those of the corresponding normals. Screening of late planted tubers gives plants with short stolons as at early plantings. The inversion of the relative stolon lengths for the 12th April planting was found to be due to frost damage. Bolters appear to be slightly more resistant than normals to frost and, as a result, the stolons were coming from the primary shoots of the bolters, but only from laterals on the normals. Thus the stolons of the normals

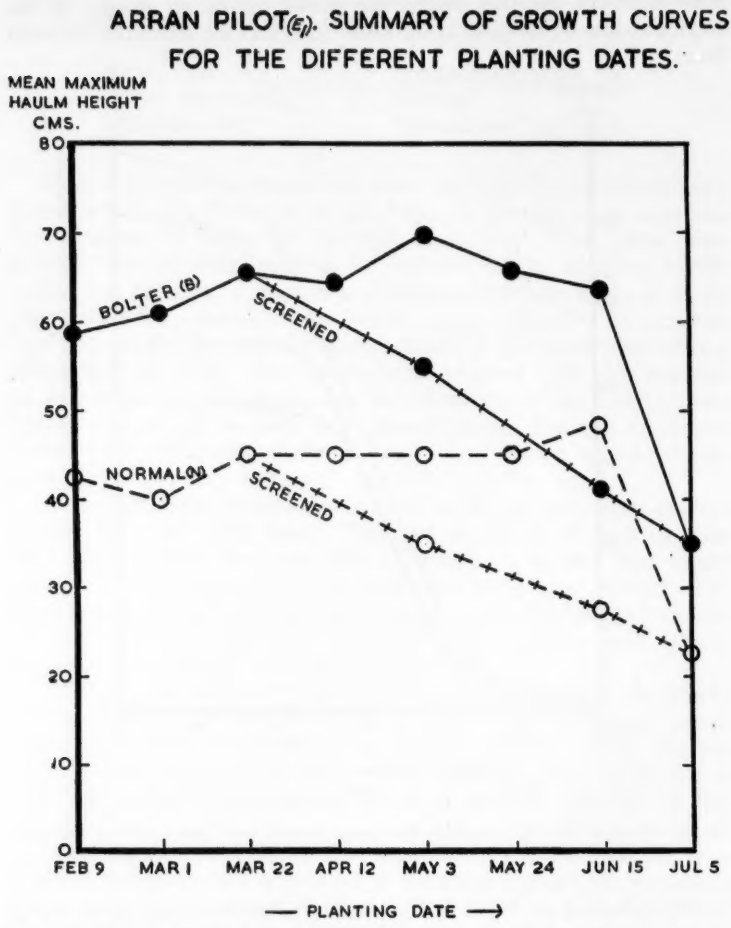


FIG. 2*b*.—The mean maximum haulm height is shown for normals (N) at the time of their maturity and the heights of bolters (B) at the same time.

TABLE 1

Length of growing period of Arran Pilot (N) when planted at different times

Planted . . .	Feb. 9	Mar. 1	Mar. 22	Apr. 12	May 3	May 24	June 15	July 5
Matured (lifting time) .	July 25	Aug. 3	Aug. 12	Aug. 20	Aug. 26	Aug. 30	Sep. 14	Sep. 23*
Growing period (days)	166	156	143	130	115	98	91	...

* Lifted immature.

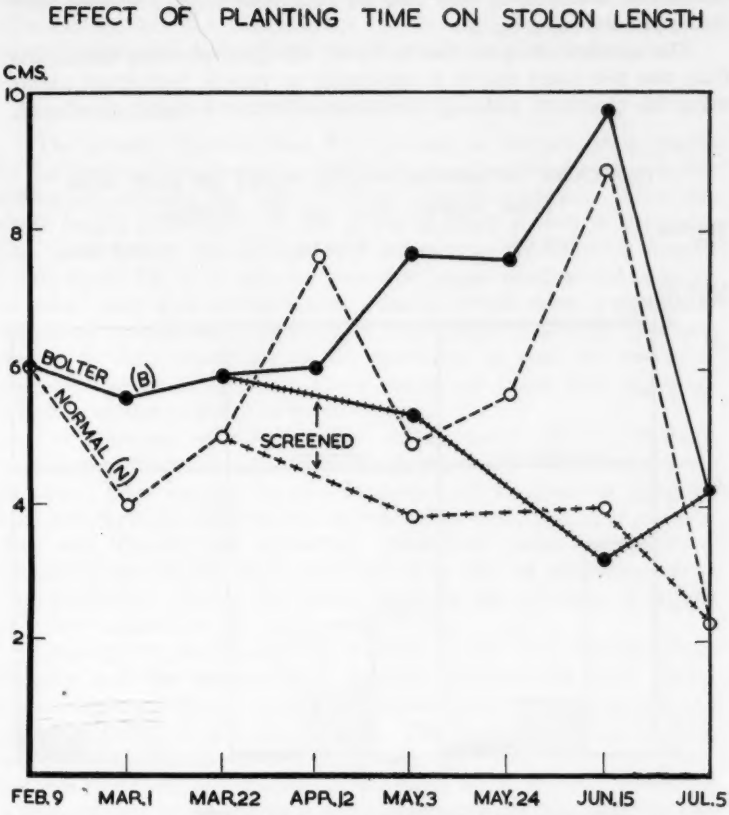


FIG. 3.—Variation in the mean length of stolons with planting time. Mean = plot mean of all stolons bearing tubers of over 10 gms. weight. (For details of screening see text.) Data on screening is available for the May 3rd and June 22nd series. The normal (N) screened and bolter (B) screened lines show the effect of the lengthening photoperiod which is received by the March 22nd series. Lifting time is as on fig. 2*b* for the various planting times.

would be produced later and therefore under longer days. At earlier planting dates the stolons came from laterals on both normals and bolters.

Secondary characters

(a) *Flowering*.—The appearance of some plants with flowers in a crop is often the earliest indication of the presence of abnormal plants, but it is not a satisfactory indication, for flower production is under the control of several environmental factors. In Arran Pilot, flower

production is extremely poor and on 23rd June 1948, a scoring gave the results shown in fig. 4.

The number of open flowers is, for all three planting times, less than one per plant and it is impossible to classify individual plants using this character, although the mean difference is highly significant.

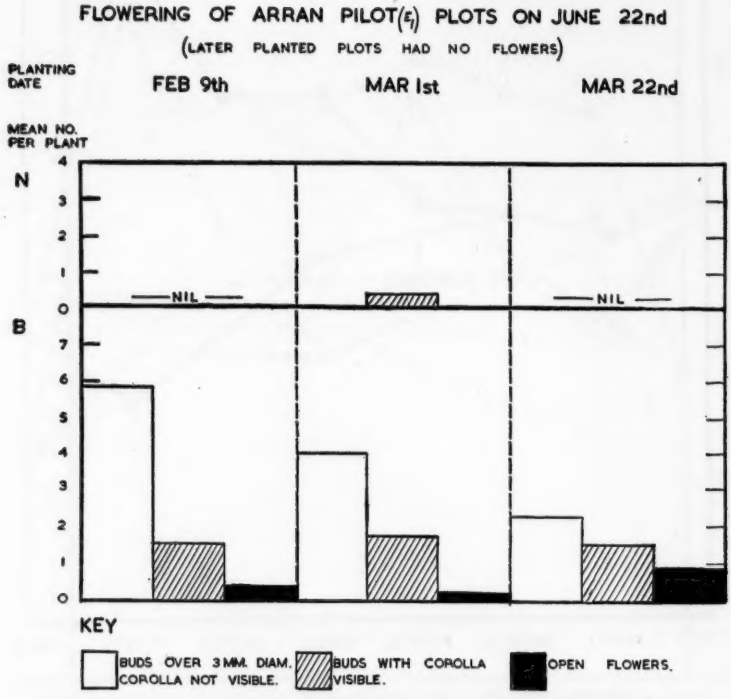


FIG. 4.—Histograms of mean flower production per plant on the plots of Arran Pilot (E_1) showing flowers on 22nd June. Flowering was at its peak at this time and none of the other plots of this variety flowered at all.

(b) *Foliage.*—In the Gladstone series again, it was noticed that the bolters showed downward curling of the leaf margin and interveinal crinkling. Samples were sent to Dr K. M. Smith, Cambridge, to test for freedom from virus and only 3 of the normals and 3 bolters gave a positive test for potato virus X. There was no difference in the appearance of the foliage of the infected bolters and the virus-free bolters, all of which showed the leaf curling not present in the normal.

(c) *Storage.*—It was noted that the bolter (B) tubers used for late planting, of both Arran Pilot (E_1) and Gladstone (E_2) were firmer

than those of the normals. This is probably due to a delayed climacteric, but the observations of Yarwood (1946) on reduced storage losses with bolters from fungal attack are also confirmed.

Conclusions

The growth characteristics demonstrated in the preceding graphs in this section were chosen as exhibiting the most clearly recognisable differences between the bolter and the normal condition. From the small height difference over the period of rapid growth in the curve (fig. 1) and from the inversion of the relative mean stolon lengths (12th April, fig. 3) it may be seen that single scorings for presence of bolters may give untrue results. Stolon length shows a significant difference at mid-season planting, but late-planted normals approach bolters in their expression of this character, in that the stolons of normals formed under midsummer conditions (15th June planting) lengthen almost as much as bolter stolons.

For plantings of tubers before 22nd March (Early planting), scoring may be possible on difference in flowering. This was discrete in Arran Pilot but not so in Gladstone. At all times of planting, however, there is a difference in duration of growth, and early planting does not obscure this character. Behaviour under conditions of increasing day-length differs therefore from that of permanent short day treatment. Under the latter condition the duration of growth of bolters approaches that of normals (Hawkes, 1947).

Covering of the plants was confined to the late afternoon and evening and the screens were removed half-an-hour after sunset. Each screen consisted of a wood frame covered with roofing felt and of size sufficient to span two plants. The forcing influence of these screens is noticed in the haulm growth (3rd May and 15th June) but this effect was not reflected in the mean stolon length. From this it appears that stolon length is a character strongly influenced by day length and less so by the other environmental factors to which the haulm may be subjected.

3. EVIDENCE FROM GRAFTING

In view of the uncertainty attached to the results of scoring progeny for the bolter reaction, it has been necessary to adopt an indirect approach to the problem of the *nature of the change normal to bolter.*

To throw light on the mechanism by which the apparently discrete change affected, in a pleiotropic manner, numerous characters in the plant, the following grafting experiments were made.

At the beginning of March 1949 six normal (N) and six bolter (B) tubers of virus-free stock seed of the variety Gladstone (E_2), selected for evenness of size, were halved longitudinally and the halves planted rose-end uppermost in pots in the greenhouse. When the leading shoots were approximately 20 cm. tall, they were reduced to one

per plant, the one chosen being as near to the rose end as possible. The plants were then placed in sets of 4, each set consisting of the two halves of a normal tuber and the two halves of a bolter tuber. The shoots were then intergrafted as shown in fig. 5.

The two self-grafted halves $\left(\frac{N}{N} \text{ and } \frac{B}{B}\right)$ then provided controls for each normal and bolter tuber, variation due to grafting being equated by the self grafting of these control pieces. The choice of sprouts from tubers of similar size and from similar positions reduced variation in vigour of the sprout due to differences in the physiological background.

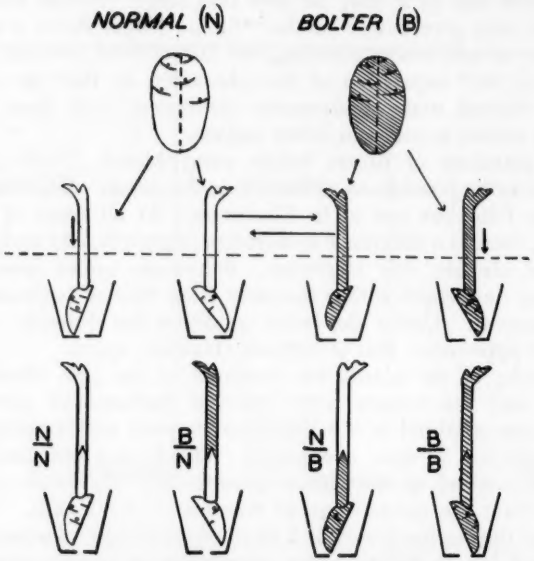


FIG. 5.—Diagram showing the method of cross grafting using sprouts from half tubers as controls. Control halves were self grafted to make any effect due to grafting a property of the whole series.

The plants were grown on in ten-inch pots in the greenhouse until the end of May and were then planted in a single row 90 cm. apart in the row, on low hills (to aid excavation). At all times the graft union was kept above soil level and the only haulm allowed to develop was that from the original scion. The plants were lifted in mid-July and the observations are summarised in table 2.

The act of grafting reduces tuber development and the yields are therefore small compared with field grown plants. The figures are the means of observation of five of the original six sets. Data from one set was discarded on the evidence of abnormal behaviour of the $\frac{B}{B}$ control plant of this set, which behaved as if it were a normal plant.

This indicated that the original supposedly bolter tuber was in fact normal and invalidated the results from this set of 4 plants.

As a general conclusion it may be seen that the scion is the more important factor in defining the type of plant which shall develop. This is shown by the figures for tubering efficiency $\left(\frac{\text{weight of tubers}}{\text{weight of top}}\right)$,

TABLE 2

Summary of data on grafted plants. Variety Gladstone

Condition $\left\{\frac{\text{Scion}}{\text{Stock}}\right.$	$\frac{\text{Normal}}{\text{Normal}}$	$\frac{\text{Normal}}{\text{Bolter}}$	$\frac{\text{Bolter}}{\text{Normal}}$	$\frac{\text{Bolter}}{\text{Bolter}}$	Standard Error of mean	P *
Mean tuber yield per plant in gms.	226	266	102	181	*60·5*	0·20
Mean number of tubers over 10 gms. weight	10·3	10·8	5·3	16·0	*1·21*	0·20
Mean stolon length in cms. .	10·7	9·1	10·1	22·1	*0·96*	0·05
Tubering efficiency : Mean $\frac{\text{tuber}}{\text{top}}$ weight × 100	**10·8**	**14·8**	**1·6**	**4·4**	*4·5*	0·05
Mean number of open flowers per plant (two observations)	2·3	0·8	9·3	12·4	*2·64*	0·05

Mean = Mean of 5 replicates.

P * = The value of the probability that the variation due to treatment is greater than the variation due to error.

the weight of top compared with the weight of tubers being very large with bolter scions. This conclusion is also true of tuber yield and freedom of flowering. The low yield of the $\frac{B}{N}$ combination is attributable to the lower vigour of the normal stock, relative to the bolter, coupled with the low tubering efficiency of the bolter scion. However, the figures for stolon length indicate that in all combinations in which the normal condition is present, $\left(\frac{N}{N}, \frac{N}{B}, \frac{B}{N}\right)$ the stolons are short. The short stolon condition of the normal does not appear to be altered with the bolter as scion, but the normal scion can affect the stolons of the bolter stock.

Before proceeding to the genetical results two experiments will be mentioned although they both yielded negative results.

Graft transmission of a virus-like particle

Of the tests for the presence of viruses, that of transmission by grafting is considered most conclusive (Bawden, 1943, p. 53). No evidence for such transmission of bolting has, however, been obtained by the author although some four hundred of the daughter tubers of $\frac{B}{N}$ grafts have been grown on for observation at various times.

Time of tuber development.—To test the possibility that the time of tuber initiation and the position of the tuber on the parent plant might influence the behaviour of the plant arising from the daughter tuber, approximately 1300 tubers were grown from 80 mapped plants. Three varieties, Arran Pilot (E_1), Gladstone (E_2) and Doone Star (E_2) were used for this purpose. Seven abnormal plants arose from this series and were identified as bolters (B). There was, however, no correlation between the appearance of these bolters and the length of stolon, tuber weight, or time of development (assessed as height up the stem of the point from which the stolon arose).

4. BREEDING RESULTS

Breeding results are summarised in fig. 6. This figure shows the frequency of bolters (B) from crosses using normal (N) and bolter (B) parents. The normal maturity classification of the parents is shown. To relate the families, those of common parentage are joined by dotted lines. The slope of the lines so produced is thus a function of the condition of the two parents, that is whether they are normal N or bolter B. Along the abscissa are marked points of varying amounts of bolter expression in the parentage. The order is arbitrary, but it has been chosen to show that the bolter used as male parent produce more bolters in the progeny than when used as female parent (M.M.Lines). The data on which the M.M. 1946 line is based was kindly supplied by Mr S. H. Revell, my predecessor in this work. This is the mean of scorings made from tubers of families, sown in the previous year, 1945. Replicates of each seedling were grown at Merton lat. 51° 25′ (John Innes Horticultural Institution) and Edinburgh lat. 55° 51′ (East of Scotland College of Agriculture). Mr Revell found that his scoring for *degree of bolting* when using the character leaflet size, flower number, maturity, vigour, length of stolon and type of tuber was the same at the two centres. The other values on fig. 5 have been compiled from the author's data on scorings made in 1948 at Bayfordbury lat. 51° 47′ (John Innes Horticultural Institution). The characters used were earliness of maturity (E), amount of flowering, stolon formation (the classification of Driver and Hawkes (1943) being used as a basis) and tubering, using the $\frac{\text{tuber weight}}{\text{top weight}}$ formula as in section 3.

Method of scoring.—From the evidence of previous results in this paper, on the influence of environment on the appearance of bolters and normals, it is clear that it is difficult to produce a satisfactory definition of a bolter (B) for scoring purposes. Carson and Howard (1944) classified the progeny from their potato crosses on the following basis, cultivated, somewhat wild, wild, and very wild. The *wildness* referred to is a generalisation for the appearance of short-day adapted species when grown under long-day conditions. It is based on the

rankness of haulm growth, amount of flowering, leaflet formation (variation in leaflet—formation is described in detail by Salaman, 1946), date of maturity, stolon and tuber formation. Driver and

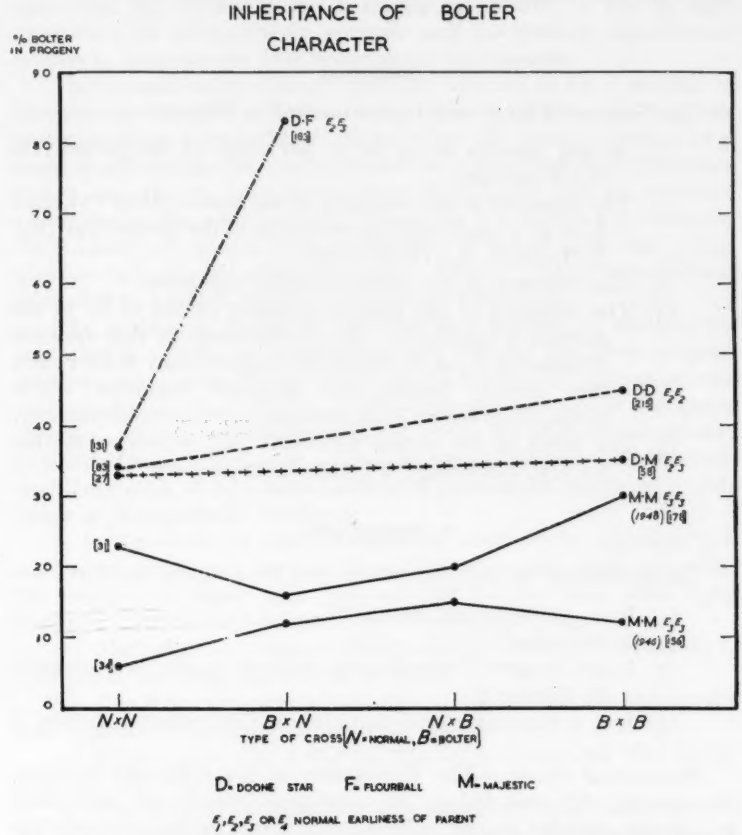


FIG. 6.—Frequency of bolters (B) in the four possible types derived from the normal (N) and bolter (B) condition of the parents. Each spot indicates the frequency of bolters from a particular family and families of common parentage are joined by a dotted line to indicate the change in percentage bolters with varying conditions of the parents. Figures in square brackets show family size.

Hawkes (1943), classify the underground parts of potato species into seven categories according to the length and number of the stolons.

In practice, the variation between trained observers is not as wide as appears possible from the complexity of the scoring methods used. Common to all the methods, is the division of the families into four groups, one of normal or cultivated types, and three of varying degrees

of bolting or wildness. It is in the partitioning between these last three groups that the variation occurs. The modified scoring method of the author was an attempt to reduce the subjective error by the use of the two statistics, $\frac{\text{tuber weight}}{\text{top weight}}$ and mean stolon length.

Conclusions

The features of fig. 5 may be summarised as follows :—

(1) Bolting appears in up to 37 per cent. of the progeny of N×N families.
(2) The influence of the condition of the parent plants (whether N or B) is small with the exception of the Doone Star (E_2) ×Flourball (E_3) (D.F.) crosses.
(3) The influence of the variety is highly significant.
(4) The influence of the time of maturity (value of E) of the parents, is significant. It is understandable that varieties of maturity E_3 or E_4 should fail to give many bolters since they already possess the principle character which distinguishes bolters from normals, that is delayed maturity.
(5) The effect of the variety Flourball (E_3) is noteworthy for the high percentage of bolters in its progeny.

5. DISCUSSION

The problem of bolting in potatoes may be analysed as follows :—

(1) Is it a physiological change ?
(2) Is it a virus ?
(3) Is the change (*a*) cytoplasmic, either in quality or quantity ? (*b*) Nuclear ?
(4) Is it a mutation (gene mutation or chromosomal change) ?

Physiological changes.—The discreteness of the difference between the normal (N) and bolter (B) conditions makes the suggestion improbable that the change normal-bolter is due to the accumulation of a tuber constituent beyond a certain threshold value. That mechanisms of this type exist in the potato has been demonstrated by Howard (1949). He found that the tubering ability of *Solanum demissum* (a short-day species) was increased where the parent plant used for the production of seed tubers had been top grafted with a long-day adapted variety of *Solanum tuberosum* (var. Epicure E_2). However, the negative result from the mapped plants experiment, section 3, and the relative constancy of the difference in other experiments rules out such a mechanism for bolting. The suggestion that it is a virus, may be discarded on the negative evidence from grafting and the positive evidence from inheritance studies.

Genetical evidence.—Until the effect of the photoperiod, in altering a number of morphological characters of the plant simultaneously, was seen to be involved in the normal-bolter change, the pleiotropic effect of this change was difficult to explain. However, it is now clear that this change might be under the control of one or more characters for photoperiodic response and the grafting experiments, section 3, demonstrates how bolter genes may operate.

The normal-bolter change therefore appears to be a nuclear or cytoplasmic mutation. Thomas (1945) showed that an extra fragment was present on a chromosome of the bolter (B) plants. Such a fragment would explain the simultaneous change in a number of characters, but it has already been shown that this change can be produced satisfactorily by a photoperiodic change and it is therefore unnecessary to produce a fragment of a constitution identical with that of the photoperiodically changing characters. Further, Revel (unpublished) has failed to confirm the presence of these fragments.

The problem is therefore narrowed to a decision of whether the mutation is nuclear or cytoplasmic and whether the gene or genes involved are separated from, or part of, the complex of genes associated with photoperiodic response in the *Tuberarium* section of the genus *Solanum*. Driver and Hawkes, (1943), review the work on inheritance of photoperiodic response in potato species and varieties and conclude that there seem to be a large number of genes involved, giving a wide range of photoperiodic reaction.)

It is impossible to state conclusively whether the inheritance is nuclear or cytoplasmic from the breeding work to date, but it resembles the problem of rogue peas (Bateson and Pellew, 1920) and rogue tomatoes (Huskins and Crane, 1930 ; Lewis, 1948, 1950).

(1) The bolter and rogue conditions all have a number of characters changed in the mutation normal to rogue.

(2) The frequency with which rogues arise germinally and bolters arise vegetatively is high. They differ in that :—

(3) Rogue peas and tomatoes arise only from seed and bolter potatoes arise vegetatively.

(4) The cytoplasmic inheritance shown in rogue peas and tomatoes by (*a*) the variation in the percentage rogue-progeny according to the position of the flower truss and (*b*) by the difference in frequency between normal and rogue and rogue and normal crosses in the percentage rogue progeny, is not apparent in the bolter problem.

(5) In rogue tomatoes the external condition, particularly temperature, during the early stages of growth, influence greatly the percentage of seedlings which develop into rogues.

The bolter condition appears, as far as evidence is available, stable in the vegetative tissue. No information is available in the bolter (B)→normal (N) mutation and the author has no definite observation on this point although he has noted the forward mutation (section 3).

The obscurity of the inheritance of bolters seems, therefore, to be due to a different mechanism from that in tomatoes and peas.

6. SUMMARY

1. Bolters occur in early varieties of potatoes as vegetative mutations to later tuber formation and abundant flowering.

2. Bolters remain later than normals when planted over the whole period from February to July.

3. The phenotype of bolters, as measured by stolon length, haulm growth and tuber weight, can be changed to that of a normal by restricting the length of day in summer.

4. Grafting shows that the scion determines the bolting behaviour rather than the stock. There is no graft transmission of a cytoplasmic determinant.

5. Each variety has a characteristic frequency of mutation, under given conditions, and there is a range in the degree of lateness of the bolters produced.

6. Breeding results show that the frequency of bolting in the progeny depends more on the variety than on the state, bolter or normal, of the parents.

Conclusion.—In its origin and determination, bolting differs from any known type of nuclear and cytoplasmic inheritance.

7. REFERENCES

ANDERSON, T. 1925. Bolters and wildings. *Board Agric. Scot.*, pub. 5, p. 43.

BATESON, W., AND PELLEW, C. 1920. The genetics of " rogues " among culinary peas (*Pisum Sativum*). *P.R.S.*, B, *91*, 186-195.

BAWDEN, F. C. 1943. *Plant Viruses and Virus Diseases.* Massachussetts : Chron. Bot.

CARSON, G. P., AND HOWARD, H. W. 1944. Inheritance of the " bolter " condition in the potato. *Nature, 154*, 829-830.

DAVIDSON, W. D. 1937. *Potato Growing for Seed Purposes.* Dublin : Stat. Office.

DRIVER, C. M., AND HAWKES, J. G. 1943. Photoperiodism in the potato. *Imp. Agric. Bureau Pub.*

FOLSOM, D. 1927. Virus diseases of the potato. *17 Ann. Rep. Quebec Soc. Protection of Plants*, 1925-26, pp. 14-29.

HACKBARTH, J. 1935. Versuche über Photoperiodismus bei südamerrkanischen Kartoffelklonen. *Züchter, 7*, 95-104.

HANSEN, H. P. 1936. Studier over Kartoffelviroser i Danmark. *Tidsskr. Planteavl., 42*, 641-681.

HAWKES, J. G. 1947. The photoperiodic reaction of potato bolters. *Emp. J. Exp. Agric., 15*, 216-226.

HOWARD, H. W. 1949. Potato grafting experiments. 1. The effect of grafting scions of Epicure on the short-day species *Solanum Demissum. J. Genet., 49*, 235-241.

HUSKINS, C. L., AND CRANE, M. B. 1930. The genetics and cytology of " rogues " in tomato, an ever-sporting character. *P. Int. Bot. C.* (5), Cambridge.

LEWIS, D. 1948. *John Innes Hort. Inst. Ann. Rept. for 1947*, pp. 6-8.

LEWIS, D. 1950. *John Innes Hort. Inst. Ann. Rept. for 1949*, pp. 11-12.

M'INTOSH, T. P. 1931. Potato virus diseases. *Scot. J. Agric.*, *14*, 86-88.

MURPHY, P. A., AND MCKAY, R. 1932. A comparison of European and American virus diseases of potato. *P.R.S. Dublin*, *20*, 347-358.

REVELL, S. H. 1947. *John Innes Hort. Inst. Ann. Rept. for 1946*, p. 9.

SALAMAN, R. N. 1926. *Potato Varieties*. Cambridge : University Press.

SALAMAN, R. N. 1946. The early European potato : its character and place of origin. *J. Linn. Soc.*, *53*, 1-27.

THOMAS, P. T. 1945. Bolters in potatoes. *Nature*, *155*, 242.

YARWOOD, C. E. 1946. Increased yield and disease resistance of " Giant Hill " potatoes. *Amer. Pot. J.*, *23*, 352-369.

Maintenance of Pure and Vigorous Stocks of the Potato. 1944. (Revised ed.) Edinburgh : Scottish Department of Agriculture.

THE CHROMOSOMES OF THE EARTHWORMS
I. THE EVOLUTION OF POLYPLOIDY

SYLFEST MULDAL
John Innes Horticultural Institution, Hertford, Herts.

Received 1.iv.51

CONTENTS

1. Introduction 55
2. Geographical Distribution and Systematic Position 55
3. Material 58
4. Chromosome Numbers in British Species . . 59
5. Systematics 68
6. Polyploidy and Parthenogenesis in *Lumbricidae* . 71
7. Polyploidy in Hermaphrodite Animals . . 73
8. Summary 75
9. References 75

1. INTRODUCTION

CYTOLOGY has been an important aid for taxonomists in plants, where breeding work on many economically important crops is now largely based on chromosome studies. But it has not been applied to animal taxonomy to anything like the same extent. The present work originated as an investigation of the breeding systems in earthworms. This group shows many remarkable reproductive features which make the results rather special and does not perhaps call for wide generalisation. The work has shown, however, that chromosome study can serve to remove systematic confusion in animals much as it has done in plants.

2. GEOGRAPHICAL DISTRIBUTION AND SYSTEMATIC POSITION

The *Lumbricidae* comprise 220 species. Of these 19 are cosmopolitan, introduced by man to almost all parts of the world; they are very robust species which often compete successfully with the local earthworm fauna. The remaining 201 are endemic; they are found only in restricted areas and are believed to be of local origin.

The family is one of the youngest in the *Oligochaeta*, and has great powers of adaptation. Their introduction into new territory often causes the disappearance of the native worms. Michaelson (1903) notes that the only genus which successfully can oppose them is *Pheretima*, the youngest shoot of the *Megascolicidae*. *Pheretima* and *Lumbricidae* share the domination of Eastern Eurasia, while the European territory belongs to the *Lumbricidae* with only one genus of another family represented—*Hormogaster*.

The distribution of the endemic species is shown on the map (fig. 1). In the eastern part of the U.S.A. a few endemic " species " are found, but it seems as if there were no really old species there, and that these forms are recently introduced and rapidly changed from their European progenitors.

The southern distribution is limited by water and dry areas. The northern line cannot be explained on present geographical conditions but it follows the edge of the ice cap during glacial periods.

After the retreat of the ice the northern area was again invaded by the more adaptable (non-endemic) species. This can clearly be

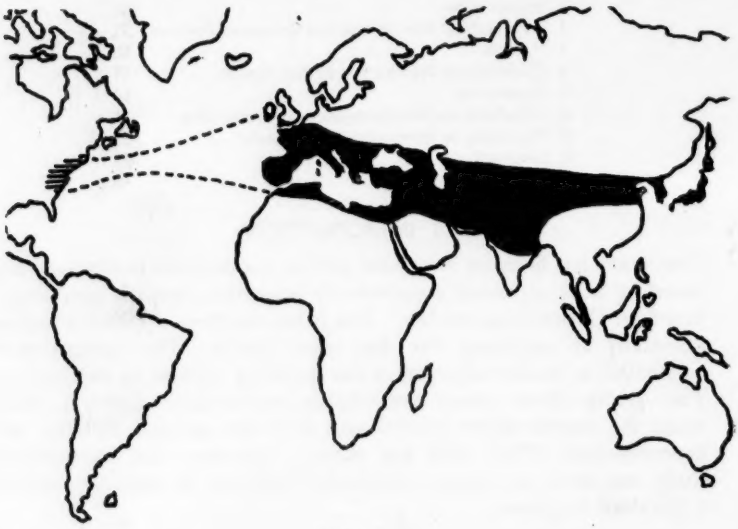


Fig. 1.

shown by the distribution of endemic species in an area which was only partly covered by the ice :—

	Endemic species	Total number of species
Germany	5	24
Czechoslovakia	20	37
Italy	40	57

It is interesting that Switzerland, although covered by the ice, has just as many endemic species (20 of a total of 38) as the ice-free Czechoslovakia. Bretscher (1903) thinks that these Swiss species should be considered as very recently developed. Michaelson (1903)

gives a different explanation. He assumes that oasis-like areas have remained ice-free between the glaciers, and that the pre-glacial endemic forms were able to maintain themselves in such places. Southern (1909) gives a similar explanation for the occurrence of *Lumbricus friendi* in Southern Ireland and elsewhere only in the Pyrenees and the Alps.

The main systematic characters of the *Lumbricidae* are the structure and position of the sexual organs, which makes it sometimes impossible to identify immature specimens. Their classification is one of the difficult problems in *Oligochaeta*. In *Das Tierreich* (1900) Michaelson divided the family into 5 genera : *Lumbricus*, *Octolasium*, *Eisenia*, *Eiseniella* and the super-genus *Helodrilus*, which was split into the subgenera *Allolobophora*, *Dendrobaena*, *Helodrilus* and *Bimastus* :—

With Testes Sacs :—
Paired . . . . . *Octolasium*
Unpaired . . . . . *Lumbricus*
No Testes Sacs :—
Spermathecal pores—
Dorsal : Gizzard long . . . . *Eisenia*
Gizzard short . . . . *Eiseniella*
Ventral : Genus *Helodrilus*—
Seminal vesicles—
4 pairs, setæ closely paired . . *Allolobophora*
3 pairs, setæ widely paired . . *Dendrobaena*
2 pairs, clitellum ending on—
segments 32/33 at the most . . *Bimastus*
segments 32/33 at least . . . *Helodrilus* (now *Eophila*)

In addition to these generic characters come the position of the spermathecal pores relative to the setæ, general size and pigmentation.

There are several difficulties in applying such a clear-cut system. The reader is referred to the discussion in Michaelson's paper of 1910, which will briefly be considered here :—

(1) *Allolobophora* and *Dendrobaena* show many intermediate forms. There are species with D-arrangement of seminal vesicles and closely paired setæ (A-character). Other species have A-arrangement of seminal vesicles, but with pigmented integument and widely paired or distant setæ.

(2) A long series of intermediate forms between *Dendrobaena* and *Eisenia* with regard to position of spermathecal pores, is given by Michaelson. The close relation here cannot be doubted.

(3) *Eisenia* and *Eiseniella*, Michaelson thinks, are so closely allied that even their complete fusion could be justified. The only distinction between them is the short gizzard of *Eiseniella* (confined to segment 17). There are, however, *Eisenia* species which have gizzards occupying only half of 18 in addition to 17, so even this character is questionable.

More recently Pool (1937) and Pop (1941) have investigated the structure of the muscle sacs in 35 species. According to their findings

there are great affinities between *Bimastus* and *Eophila* on the one hand, and between *Bimastus* and *Dendrobaena* on the other. Pop will even abolish the genera *Bimastus and Eophila* and divide these species on *Allolobophora* and *Dendrobaena*. They also claim that *Eiseniella* has no particular relationship to *Eisenia*.

We have then systematic evidence for a close relationship between the following genera :—

ALLOLOBOPHORA

EISENIELLA—EISENIA—DENDROBAENA—BIMASTUS—EOPHILA

It remains to be said that Svetlov (1924) by ignoring the genus *Helodrilus*, gave all the subgenera generic status. Cognetti (1927) followed and in the next year Michaelson accepted this classification which is the one used here. Since the present study only includes British species no attempt has been made to alter the systematics.

3. MATERIAL

Most of the material came from natural populations. Several samples have been received from Holland for comparison, but since no differences between these and the British types were found, they will not be considered separately. Samples from Switzerland were obtained through the British Museum of Natural History.

Unless especially stated the number of specimens examined have not been lower than 10. The cytological technique applied will be described elsewhere.

Owing to the small size of the chromosomes a proper study of meiosis in spermatogenesis cannot be carried out in all earthworm species.

Oogenesis, however, has been studied in nearly all species mentioned here. It appears that the whole family can be characterised by the complete absence of major spirals on the meiotic chromosomes in the eggs. But they are apparently present in spermatogenesis—hence the great size difference between the chromosomes in male and female meiosis.

It is impossible to make an accurate comparison of chiasma frequency between the various species, partly due to the impossible task of estimating variation within individuals (in the majority of species only one egg at MI can be recovered from each animal). But it is also made difficult by the long duration of MI with the pronounced stretching of the chromosomes often leading to the complete separation of the homologues.

Apart from *Eisenia foetida* Sav. the mitotic chromosomes of the British *Lumbricidae* are small. Because one only rarely finds plates with chromosomes showing marked centric constrictions it is difficult to make accurate observations on the relative length of the chromosome arms. We shall therefore only consider gross differences in morphology and number of chromosomes. In order to reduce the text the following abbreviations will be used :—

V = large v-shaped chromosome
J = large chromosome, arm ratio less than 0·5
I = medium sized rod
v = small v-shaped
i = small rod chromosome

The somatic counts were made from mitosis in embryo or testes stained with Feulgen or acetic orcein.

4. CHROMOSOME NUMBERS IN BRITISH SPECIES

The following species and forms have been studied cytologically :—

	Source of material	2n
Genus Lumbricus (Linné) :		
rubellus Hoffmeister	Merton	36
castaneus Savigny	Wisley, Kew and Merton	36
terrestris L.	Merton	36
friendi Cognetti	Basel, Switz.	36
festivus Savigny	Wisley and Merton	36
Genus Allolobophora (Eisen em Rosa) :		
caliginosa f. typica (Sav.)	Merton	36
caliginosa f. trapezoides (Duges)	Stringstone, Somerset	36
nocturna Evans	Merton	36
terrestris f. typica (Savigny)	Groningen, Holland	36
terrestris f. longa (Ude)	Merton	36
chlorotica (Sav.)	Merton	32
icterica f. typica (Sav.)	Merton	32
icterica f. dicystes (Cernosvitov)	Merton	32
Genus Bimastus (Moore) :		
eiseni Levinsen	B.	32
tenuis Eisen	B., and Switz.	48
Genus Eisenia (Malm em Michaelson) :		
foetida (Savigny)	Merton and Kew	22
veneta f. typica (Rosa)	Plymouth	36
veneta var. Hibernica f. typica (Friend)	Kew and Merton	36
veneta var. Hortensis (Michaelson)	Oxford Bot. Garden	36
rosea f. typica (Sav.)	Merton and B.	54
rosea mut. Macedonica (Rosa)	East London	53
Genus Eiseniella (Michaelson) :		
terraedra f. typica (Sav.)	Wisley and B.	72
terraedra mut. Hercynia (Michaelson)	Tring, Herts.	72
Genus Dendrobaena (Eisen em Rosa) :		
mammalis (Savigny)	Merton and Kew	34
rubida (Savigny)	Wisley and Merton	68
subrubicunda (Eisen)	Wisley, Merton and Hertford	68
Genus Octolasium (Oerley) :		
lacteum (Oerley)	B., and Valais, Switz.	38
cyaneum (Sav.)	B., and Merton	ca 190
Genus Eophila (Rosa) :		
oculata (Hoffmeister)	Kew (Thames)	32

Note.—B., Bayfordbury near Hertford.

The different genera show great homologies with regard to chromosome shape and structure. Chromosomes with strictly terminal centromeres are not present in the examined forms, and even where a mitotic chromosome in metaphase appear to be a rod, it can be shown that during prophase a short arm is always visible. Consequently it has not been possible to apply the principle of splitting or "fusion" of the centromeres in order to explain the differences in basic numbers.

Lumbricus.—Five species of *Lumbricus* have been examined; they are all diploids with $2n = 36$.

	V	I	*i*	2*n*
L. terrestris . .	16	6	14	36
L. castaneus . .	16	4	16	
L. friendi . .	16	12	8	
L. festivus . .	14	12	10	
L. rubellus . .	10	18	8	

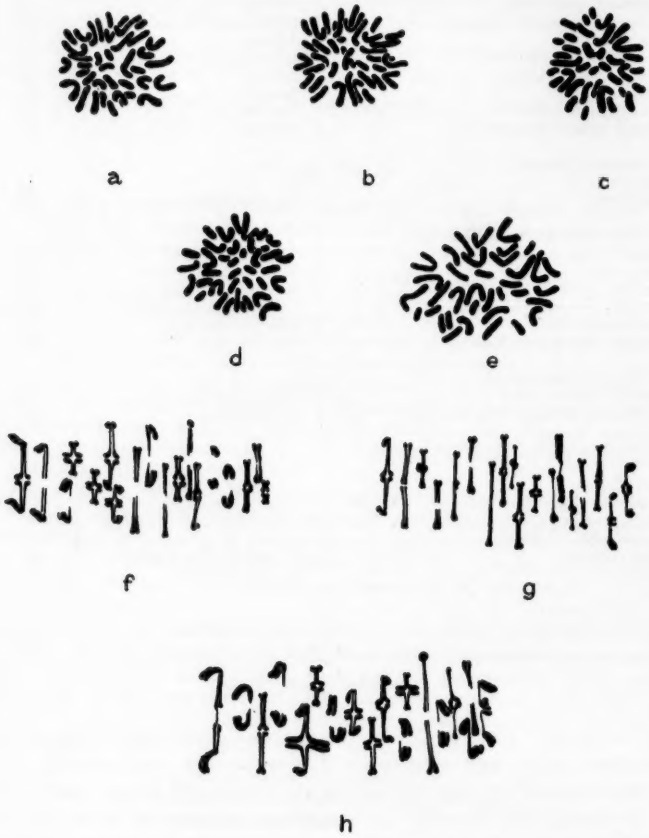


FIG. 2.—Mitosis: *Lumbricus castaneus*, embryo. (b) *L. rubellus*, embryo. (c) *L. terrestris*, embryo. (d) *L. festivus*, embryo. (e) *L. friendi*, testes.
Meiosis: MI plates from eggs of (f) *L. terrestris*. (g) *L. rubellus*. (h) *L. castaneus*. All plates × 2150.

There are no difficulties in classification in this genus. Meiosis in male and female organs occur with great regularity. The eggs are small as in the pigmented *Allolobophora* species. The receptaculæ contain generally not more than one egg at MI, except in *L. rubellus* and *L. castaneus* where two metaphase eggs were frequently found. This means that they produce cocoons faster than the others, or their cocoons contain more than one young. All the species examined are crossbreeders.

Allolobophora.—Seven forms of *Allolobophora* were examined. *A. terrestris f. typica*, *A. t. longa* and *A. nocturna* are closely related. They are the only *A*-species with dark pigmentation in England.

	J	V	I and *v*	*i*	2*n*
A. terrestris typica	2	2	18	14	36
A. t. longa	2	...	18	16	
A. nocturna	2	2	22	10	
A. caliginosa	4	2	22	8	
A. chlorotica	...	2	22	8	32
A. icterica (two forms)	...	2	16	14	

A. caliginosa has bigger cells and chromosomes than the first three species.

There is no cytological difference between the yellow and the green form of *A. chlorotica*. The former is probably a gene mutation. (Dr Kalmus has crossed the two forms, but the results have not yet been published.) *Chlorotica* and *icterica* are very similar cytologically. A specimen of *A. icterica* classified as *A. i. dicystes* could not be distinguished in chromosome shapes from the common type.

All the examined species of *Allolobophora* are obligatory cross-breeders.

Bimastus.—Two species have been examined :—

	V	J	I	*i*	2*n*
B. eiseni	10	4	4	14	32
B. tenuis	21 (V and J)			27	3*x* = 48

The latter is a triploid. The size of the chromosomes of *B. tenuis* makes it difficult to distinguish between the V and J chromosomes, but their number in the triploid agrees roughly with what one could expect if it was derived from a chromosome set similar to that of *B. eiseni*. The two species are frequently found together in the same habitat and are fairly similar in appearance. But *B. eiseni* has *closely paired setæ* and no spermathecæ. Spermathecæ are usually absent in the triploid but specimens with one or two pairs are not uncommon. This

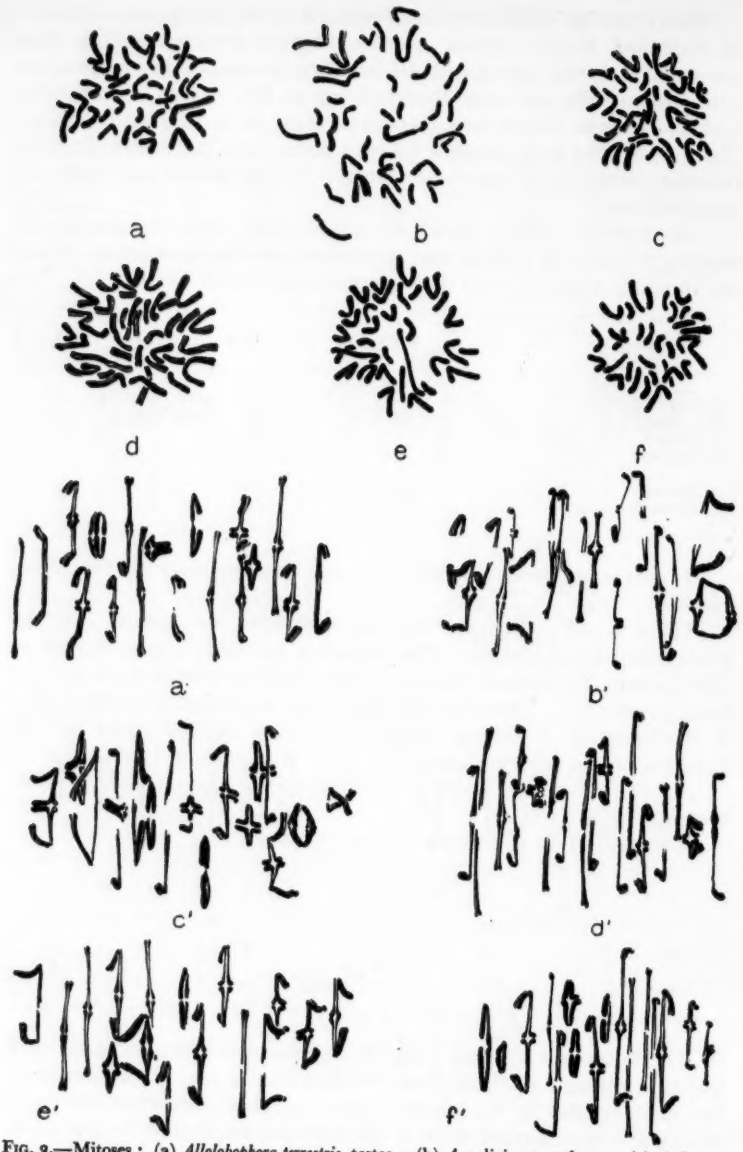


FIG. 3.—Mitoses: (a) *Allolobophora terrestris*, testes. (b) *A. caliginosa*, embryo. (c) *A. longa*, embryo. (d) *A. nocturna*, testes. (e) *A. chlorotica*, embryo. (f) *A. icterica*, embryo. All mitotic plates × 2500.

Meiosis: MI plates from eggs arranged in the same sequence as above (a'-f'). × 1750.

is surprising since the triploid *B. tenuis* has obligatory parthenogenetic reproduction.

Eisenia.—The genus *Eisenia* has two haploid numbers, $n = 11$ and 18. The low-numbered *Eisenia foetida* is the only species which allows a detailed examination of the somatic chromosomes. It has $n = 11$, as found by Foot (1896), probably the lowest number known in *Lumbricidae*.

E. foetida :—

Shapes . .	V	J	V	J	*v*	*v*	*v*	*j*	*j*	*j*	*j*
Arm ratios . .	0·7	0·4	0·7	0·3	0·6	0·9	0·7	0·3	0·4	0·3	0·2
Lengths (μ) . .	7·5	7·0	5·5	5·0	4·5	4·0	3·5	3·5	3·0	3·0	2·3

The measurements refer to late prophase.

FIG. 4.—Mitosis : (a) *Bimastus eiseni*, embryo. (b) *B. tenuis*, embryo. ×2720.
Meiosis : MI plates from eggs of (c) *B. eiseni*, and (d) *B. tenuis*. ×1970.

Since this number deviates from the usual number in the genus, it may be relevant that according to Evans and Cernosvitov (1947), *E. foetida* appears to be the only British E-species with ornamented setæ. It also differs from other species by a considerably higher fertility and in some aspects of embryo development.

Cytologically, the two "varieties" of *E. veneta* differ but little from each other. There are great morphological differences, however,

and it is very doubtful that their relationship is as close as is generally assumed :—

	J	V	*v*	*j*	*i*	2*n*
E. veneta typica . . .	2	2	4	2	26	36
E. v. hibernica . . .	8 (V and J)			2	26	

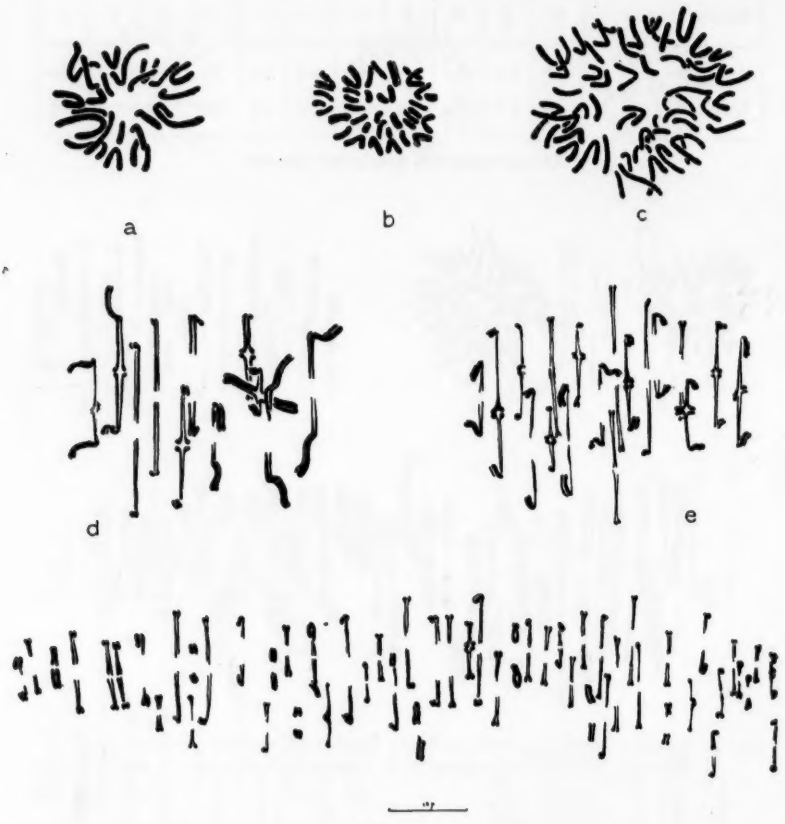


Fig. 5.—Mitosis : (a) *Eisenia foetida*, embryo. (b) *E. venata typ.*, embryo. ×1675. (c) *E. rosea*, embryo. ×2310.
Meiosis : MI plates from eggs of (d) *E. foetida*. ×2310. (e) *E. venata*. ×2310. (f) *E. rosea*. ×1170.

E. rosea has 54 chromosomes. It seems to be related to the small *Allolobophora* species, and to be a triploid with the basic number of 18.

The reproduction is obligatory parthenogenetic. The spermathecæ were never found to contain sperm and most of the specimens were practically male sterile.

The variety *E. r. Macedonica* (Rosa, 1893) has recently been found by Miss B. I. Roots in a garden in East London. Ten specimens examined by me had 53 chromosomes, $3x-1$. It seems quite likely that this loss of a chromosome is the cause of the presence of the lightly coloured glandular papillæ on the clitellum which distinguish the form from *typica* (5 specimens of *typica* collected in the same garden had the usual 54 chromosomes). This variety is previously reported from Chelsea, Kew and Wicken Fen, Cambridgeshire, and its presence in several places in England may be explained as the spreading of a clone. But it is also known from Yugoslavia (Macedonia), Switzerland and Manchoukuo, so that the chromosome loss may have occurred more than once.

Perhaps one could bridge the gap in basic numbers by examining some of the endemic species of *Eisenia*, for instance *E. alpina*. I hoped to obtain this species from Switzerland, but the three specimens received proved to be *E. rosea*, with the same chromosome number as the British type.

Eiseniella.—Two varieties of *E. tetraedra*, *E. t. typica* and *E. t. hercynia*, both had $2n = 72$, with obligatory parthenogenetic reproduction. The species is presumably tetraploid. It is polymorphic and there are only a few other species in the genus. They follow the *venata* group in distribution and may be considered as derived from the latter or a common ancestor. The generic differences have been discussed above, but one should draw attention to the frequent shift in clitellar position, which seems to be connected with limnic and aquatic habitat (Stephenson, 1930).

Dendrobaena.—Here the basic number is 17 :—

					$2n$
D. mammalis . . .	...	16 (V and J)	18 (I and *i*)	...	34
D. rubida . . .	2V	6*v*	10*j*	16*i*	34
D. subrubicunda . .	...	4 (V and J)	30 (*v* and *j*)	34*i*	68

The latter two are closely related. The tetraploid *subrubicunda* may be of recent origin since it has usually from 1 to 4 quadrivalents at MI in the eggs (Darlington, 1932). Some authors consider *subrubicunda* as a form of *rubida*. They are both widely distributed, often occurring together. *Subrubicunda* is the most common of the two.

Octolasium.—A diploid and a decaploid species of *Octolasium* have been examined :—

O. lacteum . . . 2J 14V 22*i* $2n = 38$

The chromosome shapes of *O. cyaneum* are not sufficiently clear to be analysed, but they are probably like those of *lacteum*. N = 19

is the highest basic number in the family, and the highest somatic number is also found in this genus : *O. cyaneum* with $2n = $ ca. 190. This number varies from egg to egg within 15-20 chromosomes. This variation may, of course, be due to errors in counting such high numbered plates, but considering the high frequency of abnormal divisions (micronuclei) found in somatic tissue, it seems more likely that there is a real variation.

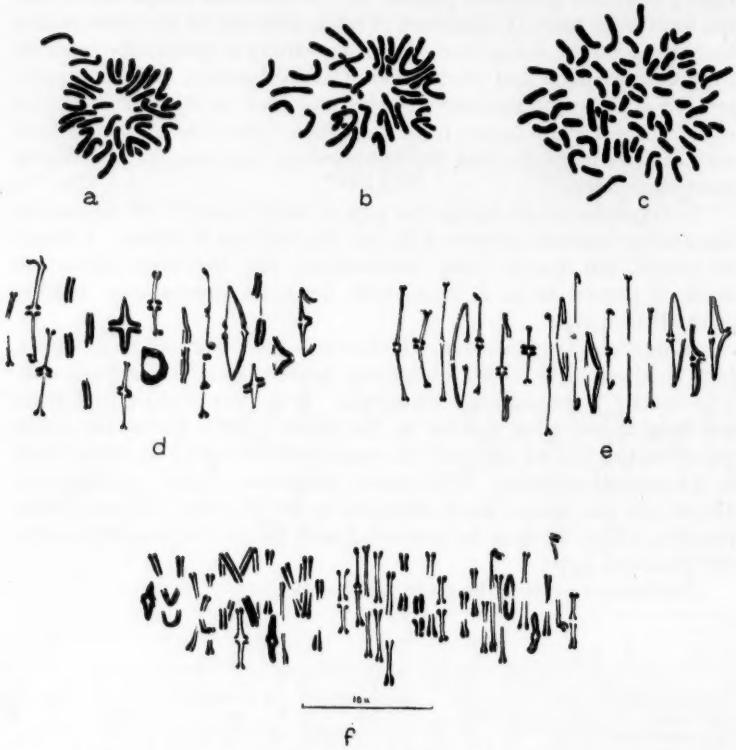


FIG. 6.—Mitosis : (a) *Dendrobaena mammalis*, testes. (b) *D. rubida*, embryo. (c) *D. subrubicunda*, embryo. ×1830.
Meiosis : MI plates from eggs of (d) *D. mammalis*. ×1830. (e) *D. rubida*. ×1830. (f) *D. subrubicunda*. ×1980.

The specific differences between *O. lacteum* and *O. cyaneum* are relatively slight. The latter should thus be considered as a decaploid. Some clones may have numbers nearer the octoploid. Both the examined species have obligatory parthenogenetic reproduction (apomictic) and reproduce with normal speed in isolation. The amount of sperm found in *O. cyaneum* is very small, but the diploid *O. lacteum* may have a fair amount of apparently normal sperm.

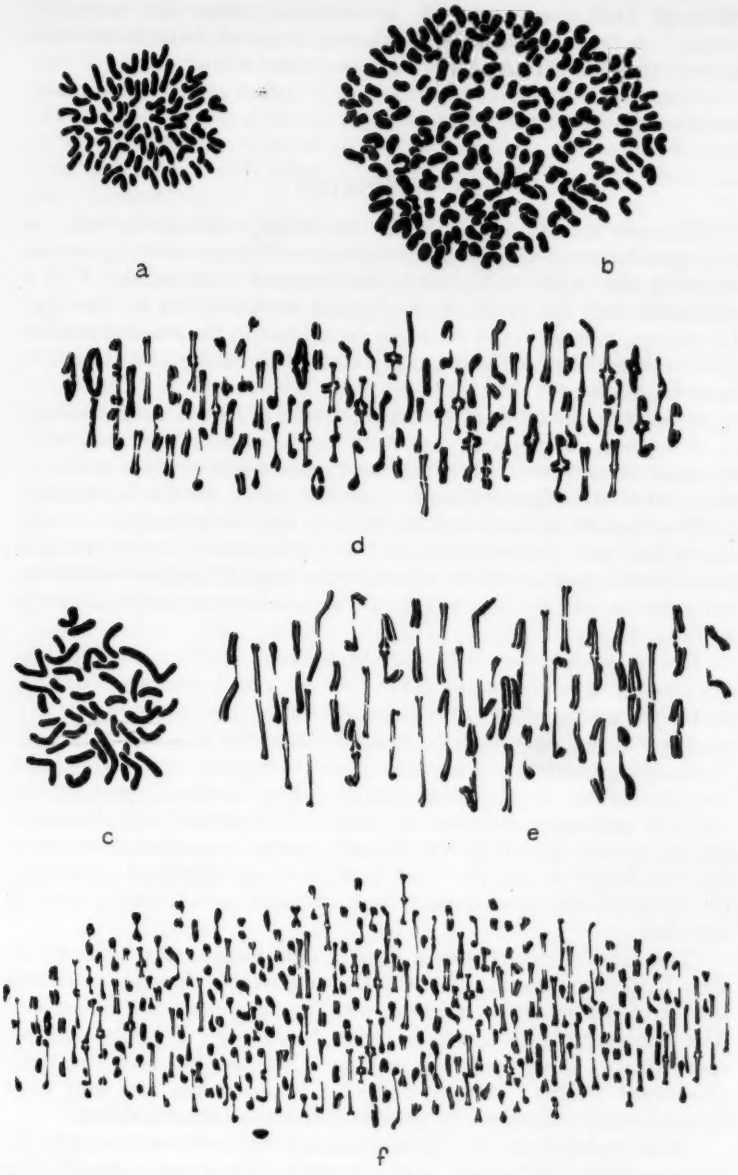


FIG. 7.—Mitosis: (a) *Eiseniella tetraedra typ.*, embryo. (b) *Octolasium cyaneum*, embryo. (c) *O. lacteum*, embryo. ×2500.
Meiosis: MI plates from eggs of (d) *E. tetraeda*. ×2500. (e) *O. lacteum*. ×2500. (f) *O. cyaneum*. ×1260.

Although both species possess spermathecæ, these are invariably empty. A few specimens of *O. lacteum* received from Switzerland showed identical chromosome conditions to the British ones.

Eophila.—One damaged specimen of *E. oculata* gave no good plates, but the number seemed to be $2n = 32$.

5. SYSTEMATICS

Contrary to the *Megascolicidae* the family *Lumbricidae* may be distinguished cytologically by the absence of late meiotic prophase stages in the ovary and possibly also internal fertilisation. This is connected with the presence of a special receptaculum for the eggs. In analogy, meiosis in the male line takes place in the seminal vesicles. Species where these vesicles are reported to be totally absent need to be re-examined.

Since only one-tenth of the known species of *Lumbricidae* is included in this study, no attempt has been made to alter the commonly accepted classification. But the survey has revealed some cracks in this system and suggested some points of attack for the taxonomist.

Five haploid numbers are recorded in the British species : 11, 16, 17, 18 and 19. *Lumbricus* ($n = 18$) and *Octolasium* ($n = 19$) are both well defined genera. If we combine the haploid numbers from the other genera with the generic affinities discussed above, we get a certain agreement.

But in *Allolobophora* both the chromosome number and external morphology agree in singling out *A. chlorotica* and *A. icterica* as a distinct part of the genus. They have their first dorsal pore in the furrow 4/5, whereas its usual position in the others is further back, in 11/12 or 12/13. They are pigmentless and have three pairs of spermathecæ. Two pairs are the normal in the other species. The " active " part of the clitellum (segments with puberty tubercles) is considerably longer in the 32 than in the 36 group although there is a causal connection between length of clitellum and number of spermathecæ. Anyhow, the clitellum ends further back in *A. chlorotica* and *A. icterica* than in the others.

The close affinity between *Eophila* and *Bimastus* are reflected in their haploid numbers. But it is also remarkable that the examined species of *Eophila* shows affinities with the 32 group of *Allolobophora*. The unpigmented *E. oculata* has first dorsal pore at 4/5, 3 pairs of spermathecæ, closely paired setæ, long clitellum—but only two pairs of seminal vesicles. *A. icterica dicystes*, however, has also only two. In other words, there are no generic differences between them.

With regard to the two haploid numbers in *Eisenia* we have already mentioned that *E. foetida* ($n = 11$) differs considerably from other British *E*-species. The species *E. fasciata* Backlund is only a form of *E. foetida* and has the same chromosome number. The triploid apomict *E. rosea* has the same haploid number as the *veneta* group ($n = 18$),

but shows also great affinities to *Allolobophora caliginosa* ($n = 18$). Of particular importance is the intermediate position of the spermathecal pores, the thickening of the anterior septæ and pigmentation (see further Evans and Guild, 1947; Julin, 1949). A relationship between *Eisenia* and *Eiseniella* may find support in their basic numbers. It is suggested that some of the numerous forms of *Eiseniella tetraedra* may differ from each other in degree of ploidy or chromosome loss as in *E. rosea*.

Dendrobaena shows affinities with three genera: *Eisenia*, *Allolobophora* and *Bimastus*, and has the intermediate haploid number 17.

In order to understand how these haploid numbers have developed we have to consider some general points about the evolution of the *Oligochaeta*.

The chromosome numbers of 14 species of *Oligochaeta* have been reported by earlier workers. Of these only two are from *Lumbricidae*, *E. foetida* ($n = 11$), Foot (1896) and *L. terrestris* ($n = 16$), Calkins (1895). The latter is incorrect.

The *Oligochaeta* have presumably arisen from the aquatic and bisexual *Polychaeta*. The latter are not well studied, but 19 species have been examined, with the highest recorded haploid number of 14. A comparison between the two groups shows that the chromosome numbers in the *Oligochaeta* are on the average higher than in the present *Polychaeta*:—

Haploid numbers	.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	—	—	—	—	28
Polychaeta	.	.	2	1	5	3			4			1	1		2									
Oligochaeta	.	.						1			2	1	1				9	3	11	2				1

It seems reasonable then to assume that there has been a general tendency towards increased chromosome numbers in the terrestrial annelids. The reason for this may be sought in the development of hermaphroditism with the subsequent release of variability in chromosome number following the loss of sex chromosomes.

The lowest numbers reported in the *Oligochaeta* are, apart from *E. foetida* ($n = 11$):—

	n	
Branchiobdella astaci . .	8	Wendrowski (1928)
Tubifex bavaricus . .	10	Oschmann (1914)
T. rivulorum (10x) . .	$x = 10$	Gathy (1900)
Enchytraeus adriaticus . .	12	Vejdowsky (1907)

Considering the low number of species examined it seems reasonable to suppose that the haploid number 9 will also be found.

We may thus assume that our diploid species of *Lumbricidae* really are old tetraploids arisen from the basic numbers 8, 9, 10 and 11. The haploid number 11 (*E. foetida*) should then be the most primitive number known in the family, and 16 and 18 be derived by doubling

of 8 and 9. Seventeen and 19 may have arisen by the loss or gain of a centromere or from interspecific crosses of 8 and 9 and of 9 and 10, with subsequent doubling as known in many plants.

If we combine the results of this survey we may attempt to reconstruct the phylogeny of the genera by the diagram.

If this be so, the two groups of *Allolobophora* and *Eisenia* may have evolved independently in more recent times and should thus constitute

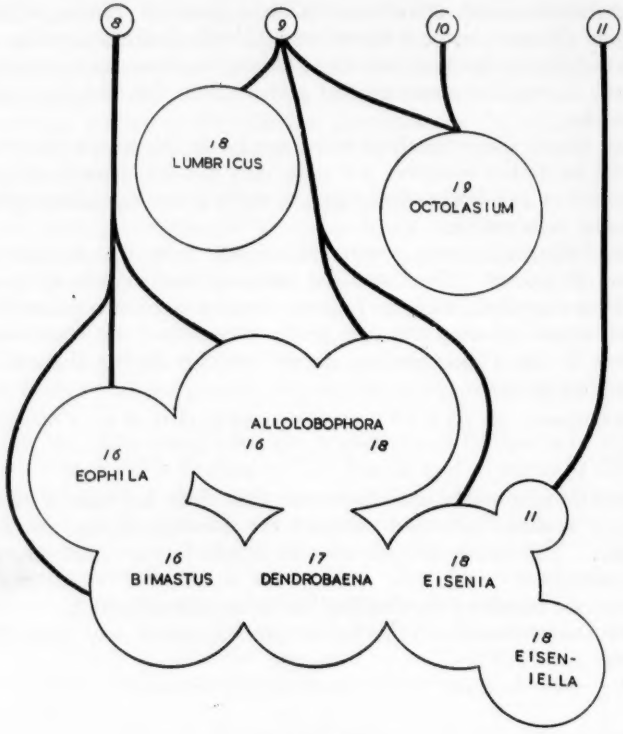


FIG. 8.

examples of convergence, *i.e.* development of similar characteristics irrespectively of ancestry, owing to lack of variety of habitat and habits. This phenomenon is regarded by systematists as common in the *Oligochaeta* (Stephenson, 1930).

From a practical point of view the present classification is perhaps convenient, but if we are to take genera as units of common descent, then *Allolobophora* and *Eisenia* ought to be divided into two separate groups or new genera. Only a more comprehensive study can decide the question of regrouping of the species in " *Helodrilus*."

6. POLYPLOIDY AND PARTHENOGENESIS IN *LUMBRICIDAE*

Of the 23 species investigated 6 are parthenogenetic and almost certainly polyploid :—

	3x	4x	10x	
Eisenia rosea (two forms) .	54	...	...	Non-sexual, Obligatory Parthenogenesis
Bimastus tenuis . . .	48	...	...	
Eiseniella tetraedra typica .	...	72	...	
Eiseniella tetraedra hercynia .	...	72	...	
Octolasium cyaneum . .	...	...	ca. 190	
Dendrobaena subrubicunda .	...	68	...	Sexual, Facultative Parthenogenesis

This clearly shows that polyploidy goes together with parthenogenetic reproduction in *Lumbricidae*.

Since there are about 220 species of earthworms described, the number of polyploids may be considerable. However, there is reason to believe that there are generic differences with regard to mode of reproduction (Evans and Guild, 1947), *viz.* cross breeding may be obligatory in *Allolobophora* and *Lumbricus*, so that polyploidy may be absent from these large genera.

Obligatory parthenogenetic reproduction found in *Lumbricidae* is apomictic. It differs, however, from the usual type of apomixis by the presence of pseudo-bivalents at MI in the eggs. These bivalents are quadrupartive in structure and show chiasma, although they consist of identical chromatids. This mode of reproduction is confined to polyploids, with the only exception of the diploid *Octolasium lacteum*.

Facultative parthenogenesis, which is also found in diploids, is automictic, *i.e.* the chromosomes pair to form the haploid number of bivalents, cross over and form chiasma. At the MI plate the centromeres divide precociously in parthenogenetic eggs, and upon division the diploid number is restored. Such eggs are frequent in unmated animals (isolated before sexual maturity) and are also common during the cold winter months. Consequently this automictic process differs from other types by the early signs of restitution.

The cytology of parthenogenesis in *Lumbricidae* will be described in detail elsewhere. In regard to morphology, there are some differences between the polyploids and their closest allies amongst the diploids. From Cernosvitov and Evans (1947) and Julin (1949) we get the following information (page 72).

Although the distribution within the limits given here in some cases certainly is left-skew (*Bimastus*), it seems to be a rule that polyploid earthworms are larger and have more segments than their allied diploid forms.

A very close affinity between the first two pairs of species may be subject to doubt, but the near relationship between the last two pairs cannot be questioned. In both these cases the species are similar even in small details, in fact, the only difference apart from those shown above is that polyploidy seems to have moved the clitellum

	Length (mm.)	Mean	Diam.	Segment no.	Mean
B. eiseni (2x) . . .	30-64	47	2-5	75-111	93
B. tenuis (3x) . .	*20-85*	*52*	*3*	*90-120*	*105*
E. venata hib. (2x) . .	23-45	34	3-4	90-120	102
E. rosea (3x) . . .	*25-85*	*55*	*3-4*	*120-150*	*135*
D. rubida (2x) . .	25-60	43	3·5	50-100	75
D. subrubicunda (4x) .	*40-90*	*60*	*4*	*60-110*	*85*
O. lacteum (2x) . .	35-160	97	4	87-165	126
O. cyaneum (10x) . .	*65-180*	*122*	*7·5*	*104-150*	*127*

forwards both in the case of *D. subrubicunda* and *O. cyaneum*. In the latter species the polyploidy is sufficiently high to show an increase in the diameter of the worm. In some cases polyploid parthenogenetic species show greater variation in length and segment number than sexual diploid species. This may be explained by the fact that the parthenogenetic species mature earlier than the sexual, and the sampling will thus be biased because of age differences.

The apomictic forms have all preserved their spermathecæ, which supports the view that selection is frozen in parthenogenetic species. This is neatly demonstrated in the genus *Bimastus*; the triploid apomict *B. tenuis* is the only British species with spermathecæ. Ribaucourt (1896) made a study of variation of pigmentation of earthworms throughout their vertical range in Switzerland. He found it to be a general rule that pigmentation increased with altitude. The only exceptions were the species *O. cyaneum* and *E. rosea*, which were of the same colour throughout their range. This is not surprising since they are both apomicts.

Vandel (1928) has called attention to the fact that if we have a bisexual and a parthenogenetic form of a single species they will usually show a deviating distribution. Usually the parthenogenetic race is the more northern one (geographical parthenogenesis). In the most typical cases the parthenogenetic form has been found to be polyploid.

It is difficult to decide whether this variation in distribution depends on parthenogenesis or polyploidy. It has been pointed out (Suomalainen, 1940; Vandel, 1940) that the distribution of polyploid parthenogenetic animals resembles that of polyploid plants. Since both the animals and the plants occur in colder and drier areas than the corresponding diploid forms, it seems that it is the polyploidy that

makes them hardier. Parthenogenesis is important as it makes the retention of polyploidy possible, and also favours the spread of the polyploid forms into new areas, since even a single parthenogenetic individual may establish a population there.

Like many diploid species, the polyploid earthworms are all peregrine, *i.e.* they are not restricted to a small area in their distribution. From Michaelson (1900) and Cernosvitov and Evans (1947) it can be seen that they are all successful and widespread species :—

Eisenia rosea typica (3*x*), cosmopolitan, the most common *Eisenia* species.

Bimastus tenuis (3*x*), the most widespread species in the genus.

Dendrobaena subrubicunda (4*x*), the most common species of its genus in Britain, but may be less common than *D. rubida* (2*x*) on the Continent. Recorded from more places all over the world than any other species.

Eiseniella tetraedra typica (4*x*) and *m. Hercynia* (4*x*) are the only representatives studied in the genus. Both are almost cosmopolitan, the latter may be less common ; it is only recorded from two localities in Britain.

Of all the recorded *Octolasium* species, *O. lacteum* (2*x* apomict) and *O. cyaneum* (10*x*) have the widest distribution. They have been reported from all over the world.

On the whole it can be safely concluded that within their genera the polyploids are all the most successful and widespread species.

7. POLYPLOIDY IN HERMAPHRODITE ANIMALS

The present evidence of polyploidy can be taken to support Muller's arguments for the rarity of polyploidy in animals as opposed to plants. More than half of the higher plants are polyploids (Müntzing, 1936).

Based on the discovery of the X/autosome balance as a means of sex determination in *Drosophila*, Muller (1925) pointed out that in bisexual organisms polyploidy will inevitably upset the sex chromosome mechanism, since it automatically abolishes pairing of dissimilar chromosomes. Thus a tetraploid of an originally XY-XX form will have the composition XXYY-XXXX, and the gametes of the heterogametic sex should be XY due to the X's and Y's forming separate bivalents at meiosis. Pairing between two tetraploids would thus lead to only males and there would be no chance of starting a tetraploid race. Muller further considered that triploidy was a necessary step between diploid and tetraploid forms. In *Drosophila* 3*n* females are fertile and vigorous, and when mated to diploid males they produce, apart from diploids of both sexes, females like themselves and inviable or infertile unbalanced individuals. Thus the number of triploids will tend to decrease in each succeeding generation.

Muller concludes : " If the present theory is correct, it may receive two lines of support from comparative cytology. For amongst groups of plants the sporophytes of which are always dioecious and have the " XY " or " WZ " type of sex determination, there should be

the same lack of evidence of tetraploidy as amongst most animals.* And amongst groups of animals like earthworms and freshwater snails, which are normally hermaphroditic, tetraploidy or even higher forms of polyploidy might occur as readily as amongst plants."

Now what is the evidence for polyploidy in hermaphrodite animals in general? White (1940) examined what was known of the Flatworms, *Oligochaeta*, Leeches and Pulmonate Molluscs and concluded that the evidence was only slight and that polyploidy could not have played anything like the important role it has in plants. This statement, based on what is certainly very scrappy evidence, seems safe enough as long as one assumes (i) that cross fertilisation is obligatory in all species (in plants selfing is very common), and that (ii) parthenogenesis is absent.

Male gonads do not usually offer good material for chromosome determinations in the hermaphrodite animal groups, and never of course give any indication of the mode of reproduction. In order to answer these questions we must consider only reports describing oogenesis, as follows :—

(1) In *Turbellaria* the number of species where oogenesis has been examined is very small :—

Acoela : Only spermatogenesis examined.

Rhabdocoelida : As above, asynapsis reported.

Allocoela : *Bothrioplana semperi* Braun. Parth. : Reisinger (1940).

Tricladida : *Pylocelis nigra*. Parth. : Lepori (1948, 1949).

Dugesia gonocephala Parth., polyploidy (2*x*, 6*x*) : *D. benazzii* Lepori Parth., polyploidy (3*x*, 4*x*) : Benazzi-Lentati and Nardi (1950).

Polycladida : *Notoplana humilis* Sexual : Kato (1940).

There seems to be no reason to doubt that parthenogenesis and polyploidy are common in this group.

(2) In *Lumbricidae* cross breeding may be obligatory in the genus *Lumbricus* and perhaps also in the bigger *Allolobophora* species (Evans and Guild, 1947). But in other genera, such as *Bimastus*, in species lacking spermathecæ, self fertilisation may occur unless parthenogenesis (with or without pseudofertilisation), is obligatory. Facultative automixis and apomixis have been established in a number of species (Muldal, 1949). It is unlikely that parthenogenesis and polyploidy should be restricted to only one family of the *Oligochaeta*.

(3) In *Hirundinea* there are only 4 accounts of oogenesis (in Makino's list), all apparently with sexual reproduction. Two species have $n = 8$, one has $n = 13$ and one $n = 16$. The latter may be a tetraploid, but the data are too meagre to allow any conclusion.

(4) In Pulmonate Molluscs there are a lot of counts from spermatogenesis (after 1930), but only in three instances has oogenesis been examined. These reports give the haploid numbers 18, 27 and 31. In this group there are probably

* However, there are some examples of natural polyploidy in dioecious plants :

Antennaria carpathica .	3*x*	*Rumex dioica* . .	6*x*	*Salix aurita* . . .	4*x*
Elodea canadensis . .	4*x*	*Urtica dioica* . .	4*x*	*Salix cinerea* . . .	4*x*
Fragaria elatior . .	6*x*	*Salix lucida* . . .	4*x*	*Salix Andersoniana* . .	6*x*

Westergaard (1940) produced a stable tetraploid strain of the dioecious *Melandrium album*, and criticised Muller's theory. The diploid XY-XX system was transferred to the stable XXYY-XXXX system. This is probably an example of strong male determining power of the Y-chromosome.

great technical difficulties shown by the conflicting reports from spermatogenesis. The value of Perrot's work is greatly reduced by the absence of information on the modes of reproduction. His report of sex chromosomes in 5 species seems doubtful on *a priori* grounds, and is best explained by the work of Husted and Burch (1946) who reported the presence of 1-4 extra chromosomes in different strains of *Triodopsis fraudulenta*. Selfing or parthenogenesis is reported in very old literature, but new evidence must be put forward. It is clear that a thorough survey of breeding systems is necessary for the whole group of Pulmonate Molluscs.

From this survey it must be quite clear that there is no reason for believing that (i) cross breeding is obligatory, and (ii) that parthenogenesis is rare in hermaphrodite animals. White's conclusion that the evidence of polyploidy in hermaphroditic groups is only slight, does not seem to have a firm basis.

8. SUMMARY

1. The chromosome number of 30 species and forms of British *Lumbricidae* are given.

2. The basic numbers 11, 16, 17, 18 and 19 have been found in the family. Four of these have not been reported previously. These numbers confirm generally the systematic division of the group.

3. Polyploidy is reported from 5 genera, including triploidy, tetraploidy and decaploidy.

4. All the polyploids show parthenogenesis, a method of reproduction not previously shown in *Oligochaeta*. In all but one the parthenogenesis is of the obligatory diploid type. One diploid also had this mode of reproduction.

5. *Dendrobaena subrubicunda*, a tetraploid, like many diploid species, has facultative parthenogenesis, but is undoubtedly a case where polyploidy and sexual reproduction occur side by side.

6. All obligatorily parthenogenetic species are (i) polyploid, except one, (ii) peregrine, *i.e.* non-endemic, (iii) larger than the nearest diploids and (iv) relatively invariable. It is suggested that these four characteristics are associated with parthenogenesis.

7. A survey of hermaphrodite animal groups indicates that parthenogenesis and polyploidy may be not infrequent. Also that in them, as in dioecious groups, polyploidy is associated with parthenogenesis.

9. REFERENCES

BENAZZI-LENTATI, G., AND NARDI, O. 1950. *Caryologia, 3*, 181-199.
BRETSCHER, K. 1903. *Biol. Zbl., 23*.
CALKINS, G. N. 1895. *J. Morphol., 2*.
COGNETTI DE MARTIIS, L. 1927. *Bull. Mus. Genova, 2*, 7.
DARLINGTON, C. D. 1932. *Recent Advances in Cytology*. London.
EVANS, A. C., AND GUILD, W. J. MCL. 1947. *Ann. Mag. Nat. Hist.*, ser. 11, *5*, 14.
EVANS, A. C., AND CERNOSVITOV, L. 1947. *Lumbricidæ*. London : Linn. Soc.
FOOT, K. 1896. *J. Morphol., 4*.
HUSTED, L., AND BURCH, P. R. 1946. *Amer. Nat., 80*, 410-429.

JULIN, E. 1949. *Arkiv för Zoologi*, B. 42, No. 17, 1-58.
LEPORI, N. G. 1949. *Caryologia, 1*, 280-295.
LEPORI, N. G. 1950. *Caryologia, 2*, 301-324.
MAKINO, S. 1950. *Review of Chrom. No. in Animals.* Tokyo : Hokuryukan Publ. Co.
MICHAELSON, W. 1900. *Das Tierreich : Oligochaeten.* Berlin.
MICHAELSON, W. 1903. *Die geogr. Verbreitung d. Oligochaeten.* Berlin.
MICHAELSON, W. 1918. *Zool. Jahrb., System., 41.*
MULDAL, S. 1949. *Proc. Linn. Soc., 161*, 116-118.
MULLER, H. J. 1925. *Amer. Nat., 59*, 346-353.
MÜNTZING, A. 1936. *Hereditas, 21*, 263-378.
PERROT, P. 1937. *Rev. Suisse Zool., 45.*
PERROT, P. 1938. *C.R.A.S.*, 207.
POOL, G. 1937. *Acta Zool., 18*, 1-110.
POP, V. 1941. *Zool. Jahrb., Syst., 74*, 5, 6 Jena, 487-519.
REISINGER, E. 1940. *Chromosoma, 1*, 531-553.
RIBAUCOURT, E. DE. 1896. *Rev. Suisse Zool., 4.*
SOUTHERN, R. 1909. *Proc. Roy. Irish Acad., 27* B.
STEPHENSON, J. 1930. *The Oligochaeta.* Oxford : Clarendon.
SUOMALAINEN, E. 1940. *Ann. Acad. Sci. fenn.*, A, *6*, 1-30.
SUOMALAINEN, E. 1940. *Ann. Acad. Sci. fenn.*, A, *7*, 1-144.
SVETLOV, P. 1924. *Bull. Inst. Rec. Biol. Perm., 2.*
VANDEL, A. 1928. *Bull. Biol., 62*, 164-281.
VANDEL, A. 1940. *Bull. Biol., 74*, 94-100.
VEJDOVSKY, F. 1907. *Neue Untersuch. u. d. Eireif. u. Befruchtung, Prag.*
WESTERGAARD, M. 1940. *Dansk Bot. Ark., 10*, 5, 1-131.
WHITE, M. J. D. 1940. *Nature, 146*, 132.

HETEROCARYOSIS IN WILD PENICILLIUM

J. L. JINKS

Agricultural Research Council's Unit of Biometrical Genetics, Department of Genetics, University of Birmingham.

Received 5.v.51

HETEROCARYOSIS is the association in all the cells of a lineage of nuclei of unlike genetical constitution. These nuclei are presumably haploid and do not normally fuse, although they may do so prior to sexual reproduction, as in *Aspergillus nidulans* where sexual and heterocaryotic systems co-exist (Pontecorvo, 1946). The work described so far in this field has dealt with heterocaryons derived from two homocaryons that differ by induced biochemical mutations, which in many cases are lethal in the homocaryotic state on a minimal medium (Beadle and Coonradt, 1944 ; Pontecorvo and Gemmell, 1944*a* and 1944*b* ; Sansome, 1946). These studies on artificial heterocaryons have led to much progress in the investigation of gene action and biochemical syntheses. They have also suggested that heterocaryons may have an importance in nature, but unless the " dual phenomenon " (Hansen, 1938), whose genetical and biological significance has not yet been made clear, is to be regarded as ascribable to heterocaryosis, we have had no evidence so far of the occurrence or operation of the system in wild fungi. The present investigations were undertaken with a view to supplying such evidence.

A recent claim has been made by Papazian (1950) to have produced heterocaryons from pairs of wild homocaryons of *Schizophyllum commune*. His claim is not, however, borne out by his observations. Twenty single hyphæ were taken from the so-called heterocaryon, but in no case was it possible to show the presence of both original homocaryons in the growth from any one hypha, although the tests for the homocaryons were quite adequate.

ORIGIN OF MATERIAL

Sixteen examples of the *Asymmetrica* group of the genus *Penicillium* were obtained from air, soil and various infected materials, and maintained on a minimal medium, Czapek agar, of following composition :—

$NaNO_3$	2·0 gms.	KH_2PO_4	1·0 gm.
KCl	0·5 gms.	Sucrose	30·0 gms.
$MgSO_4 7H_2O$ . . .	0·5 gms.	Agar	20·0 gms.
$FeSO_4 7H_2O$ . . .	0·01 gms.	Water	1000 c.c.

at 25° C. They were propagated by single hyphæ. The sources of the various wild isolates are given, along with relative growth rates and other relevant data, in table 1.

TABLE 1

The 16 wild isolates of the Asymmetrica *group of the genus* Penicillium

No.	Date of isolation	Source	Results	Relative growth rate
1	7.11.50	atmosphere	*Heterocaryon*	70·6
2	7.11.50	atmosphere	...	58·1
4	11.11.50	atmosphere	*Saltated*	72·2
5	12.11.50	saprophyte on capsule of *Nicotiana rustica*	*Heterocaryon*	100·0
6	23.11.50	saprophyte on apple	...	64·7
7	23.11.50	saprophyte on marrow	...	69·7
8	23.11.50	saprophyte on bread	...	91·3
9	25.11.50	atmosphere	*Homocaryon*	81·3
11	28.11.50	atmosphere	*Heterocaryon*	72·2
12	9.12.50	saprophyte on lemon	...	81·3
13	15.12.50	saprophyte on cocoanut	*Homocaryon*	81·3
14	3. 1.51	saprophyte on apple	...	69·7
15	7. 2.51	soil	...	81·3
16	7. 2.51	saprophyte on seed of *Nicotiana rustica*	...	54·0
17	7. 2.51	soil	...	86·3
18	7. 2.51	soil	...	77·2

The relative growth rates are based on No. 5, which is the most thoroughly investigated line to date. These are used because the absolute growth rates vary with each new sample of agar. Thus No. 5 has given growth rates that vary from 9·67 to 12·3 mm. per 2 days increase in diameter of its colonies. This variation necessitates a strict system of controls in all comparative work.

All the lines investigated so far have uninucleate conidia, while none have an ascosporic stage, reproduction thus being entirely asexual. No attempt has been made to classify the lines into species, as this is immaterial for the work undertaken. In any case, with the classification of *Penicillia* in its present state the species concept has little significance in this group.

BREAKDOWN OF HETEROCARYON No. 5

The method employed in the investigation of No. 5 will be described as an example of the general technique. No. 5 was obtained as a saprophyte on a capsule of *Nicotiana rustica* and isolated as a single hypha. It was single spored by streaking a drop of a dilute suspension of conidia on to a plate of filtered minimal medium. After incubating for 18 hours at 25° C. the germinated spores are visible under a low power microscope, viewed from the under-surface of the petri dish, and their individual positions can be marked on the under-surface.

They can then be dissected out by hand from above. Thirty-one colonies were obtained in this way and were found to fall into two distinct groups, A and B, distinguished by their growth rates. A grew more quickly than B but not so quickly as parent No. 5. Of the 31 single spore lines so obtained, 24 were of type A and 7 of type B. An analysis of variance showed that A and B could be regarded as homogeneous groups, and that they differed significantly from one another in growth rate. Fig. 1 shows the increase in diameter of the colonies of the single spore lines, together with the original parent, during 6 days' growth.

The ratio of the growth rates of No. 5, A and B are 100 : 62 : 36·5 based on area, or 100 : 83 : 63 based on diameter, of their colonies

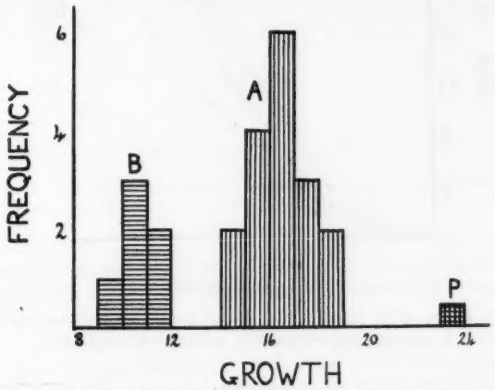


FIG. 1.—Distribution histograms of the increase in the diameter, from 2 to 8 days, of the colonies of the single spore lines of No. 5, showing the two homogeneous groups A and B, with the original heterocaryon P for comparison.

after 12 days' growth. The results from which these ratios were obtained are plotted in fig. 2. Although the absolute growth rates vary with different samples of agar these ratios have shown no significant deviation during four months.

The percentage germination of spores from A and B has been investigated at 12°, 17° and 25° C. No. 5 was also investigated at 12° C. The results of 3,800 observations are shown in table 2, and the graph for 12° C. in fig. 3. Although A grows quicker than B the reverse relation is shown by their rates of germination. There is a strong suggestion that the conidia from No. 5 are non-autonomous during germination, in that the average rate of germination bears no relation to that expected of a mixture of conidia of type A and B. Spore colour was also found to be non-autonomous for a member of this group. *Penicillium notatum*, by Pontecorvo and Gemmell (1944*a*).

As far as morphological characters and appearance of the colonies are concerned, No. 5, A and B are identical. In fact the three lines,

5, 9 and 11 which have been studied in detail are indistinguishable except by such physiological characters as rate of growth of colonies and of the germ tubes of conidia.

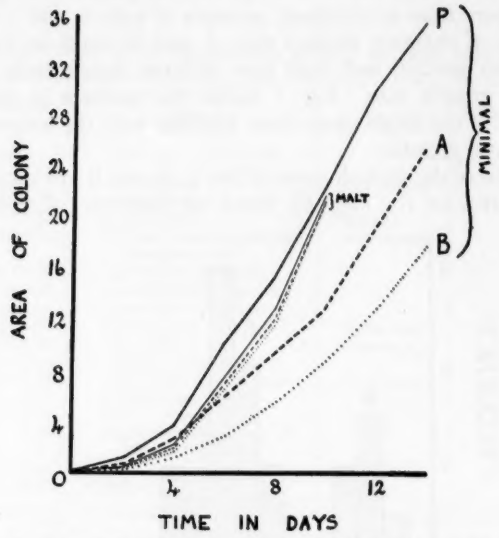


FIG. 2.—Growth curves of the homocaryons A and B and the heterocaryon No. 5 on both a minimal and a 2 per cent. malt extract medium.

TABLE 2

Comparison of the percentage germination of homocaryons A and B at 12, 17 and 25° C. and of heterocaryon No. 5 at 12° C. at various times

Time	Temperature and type						
	12° C.			17° C.		25° C.	
	5	A	B	A	B	A	B
17 hrs.	13·8	2·0	18·6	...	...	15·4	40·7
18 hrs.	...	...	...	10·0	21·0	...	...
19 hrs.	24·6	5·1	...	...	...	...	
21 hrs.	31·4	10·5	33·5	18·3	31·6	...	...
23 hrs.	41·6	23·0	41·9	...	43·2	...	...
24 hrs.	...	...	...	40·0	48·0	...	...

When grown on a 2 per cent. malt extract agar, which while being richer than the minimal medium is not complete, the growth rates of 5, A and B were indistinguishable and differed only slightly from that of 5 on a minimal medium (table 3 and fig. 2).

It appears, therefore, that we are here dealing with a system akin to the artificial heterocaryons derived from two homocaryons carrying biochemical mutations; the homocaryons growing better on an enriched than on a minimal medium but no better than the heterocaryon on a minimal medium. Such systems differ, however, from

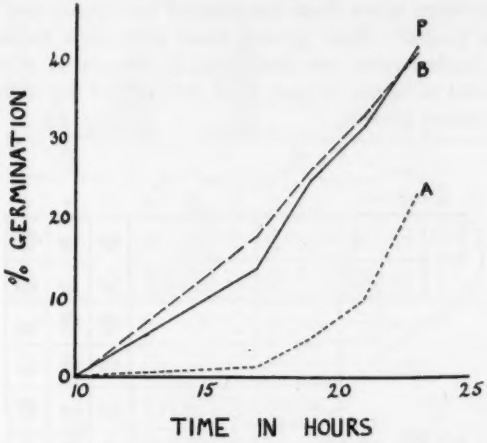


FIG. 3.—Rate of germination of spores from the homocaryons A and B and the heterocaryon No. 5, at 12° C. expressed as percentage of germinated spores against time.

TABLE 3

Comparison of the average diameters (in mm.) of the colonies of the homocaryons A and B and heterocaryon No. 5 on 2 per cent. malt extract medium and heterocaryon No. 5 on a minimal medium at 2 day intervals. The last column gives the average increment per 2 days.

Medium	No.	Time	2	4	6	8	10	Average
2 per cent. malt extract	A	2·0	9·0	19·0	...	39·0	53·0	10·2
	B	2·0	7·0	17·0	...	38·0	52·0	10·2
	5	2·0	10·0	19·5	30·0	40·0	53·0	10·2
Minimal	5	2·0	12·0	22·0	36·0	45·0	53·5	10·3

the wild heterocaryon described, in that the homocaryons derived from the wild, while growing better on an enriched medium, are capable of growing satisfactorily, albeit at a slower rate, on a minimal medium. The shortcomings of the wild nuclei are thus less drastic than the biochemical mutations, which are of course lethal on a minimal medium. Indeed, whatever the nature of their inheritance, variants of the kind we find in the wild would not be recovered as laboratory mutants by the standard technique, for in this technique only those induced biochemical variants, which fail to grow at all on a minimal medium are tested further.

RECONSTITUTION OF THE HETEROCARYON

The first twelve single spore lines to be isolated, consisting of 9 type A and 3 type B, were cross tested two at a time for heterocaryon production. Spores of each of the pair of lines to be tested were inoculated on to the same point in the agar. After 6-8 days' growth single hyphæ were taken from the edge of the colony and transferred to new agar plates. Their growth rates were then measured. The removal of single hyphæ was facilitated by the use of plain agar and by the removal of blocks of agar from the path of the colony, so as to promote a sparser growth.

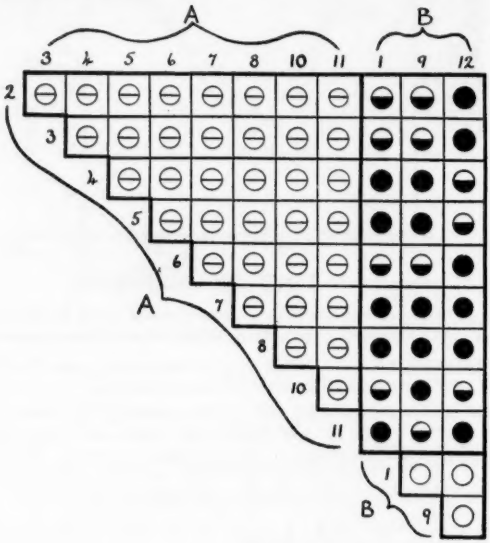


FIG. 4.—Diagram to illustrate the results of the attempts to reconstitute heterocaryon No. 5 from the homocaryons A and B. ⊖ denotes combinations giving type A growth rates, ○ type B, ● heterocaryon growth rate and ◒ combinations that gave type A growth rate on first testing but eventually gave the heterocaryon type growth after retesting.

For convenience the experiment, which involves 66 cross tests, was divided into three parts so that each part contained all three types of combinations, *i.e.* B+B, A+A and A+B. In part two, however, the B+B single hyphæ failed to grow. A fourth set of tests was subsequently undertaken to complete the observations. This comprised retests of all A+B combinations which failed to produce heterocaryons and also of the cases where the single hyphæ failed to grow in the earlier tests. The results are summarised in fig. 4. All the A+A combinations grew at the same rate as type A, and B+B combinations similarly gave only type B growth rate. In 19

out of the 27 cases of the combination A+B, the growth rate was that of the parent heterocaryon. The remaining 8 cases gave the type A growth rate. Heterocaryotic growth was ultimately obtained from these 8 A+B combinations after retesting, although as many as six attempts were necessary in some cases before this was achieved.

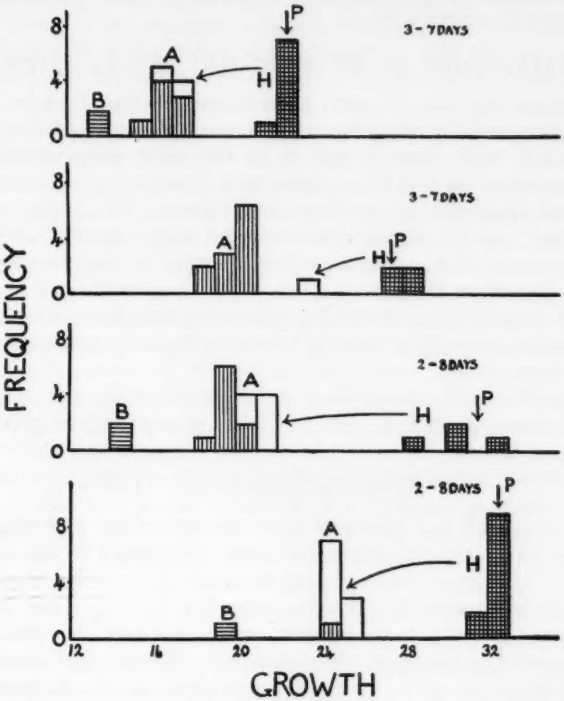


FIG. 5.—Distribution histograms of the increase in diameter of the various combinations of the homocaryon lines, during the time specified. A denotes A+A combinations, B denotes B+B combinations and H denotes A+B combinations. The unhatched columns with the arrow from H are the A+B combinations that failed to form heterocaryons. P denotes the increase in diameter of heterocaryon No. 5 under the same conditions.

In all, 57 per cent. of cases where spores of A and B were inoculated together gave heterocaryotic growth.

The actual growth rates of these various combinations are shown as histograms in fig. 5, which is the frequency distribution among the cross tests of the various growth rates observed. Thus in the first set of tests, two of the cultures of combination B+B gave growth between 14-15 mm. in diameter while one of type A+A gave a growth of 15-16 mm. in diameter and so on. Reconstituted heterocaryons H and the original heterocaryon P, caught from the wild,

had almost identical growth rates in any one set of tests, although the absolute growth rates vary from occasion to occasion, presumably with the medium. The unhatched histograms with the arrow from H are the growth of colonies of A+B combinations that failed to give heterocaryons, giving instead A growth as might be expected. The histograms marked A and B are the growths of A+A and B+B combinations respectively.

BREAKDOWN OF RECONSTITUTED HETEROCARYONS

Three of the reconstituted heterocaryons were taken at random and single spored in the same way as was the original heterocaryon. All yielded both types A and B in the same proportions, within sampling error, as did the original heterocaryon, *viz.* 20A to 7B as compared with 24A to 7B from the original. As checks, one type A+A and one B+B combination were single spored, and twelve lines grown of each. The first gave all type A and the second all type B growths. With twelve single spore growths and a nuclear ratio of roughly 3A : 1B in the heterocaryons, there was less than one chance in twenty of missing the rarer type of single spore growth had it been present.

Thus the wild parent is a heterocaryon with two genetically distinct types of nucleus, which jointly give a higher growth rate than either alone.

INVESTIGATION OF No. 11

No. 11, which was obtained from the atmosphere as a single point infection on an exposed plate, has been investigated in the same way as No. 5. On first single sporing only two of the twelve single spores plated gave measurable colonies (type C), the other ten produced microscopic growths (type D) that ceased, presumably, when all the food material in the spore was exhausted. Removal of these minute growths to 10 per cent. malt extract resulted in little further growth and they finally died. The culture that was single spored was transferred to a new plate, and the transferred colony, on a further single sporing, gave 8 normal colonies, type C, and 2 type D, which even though plated directly on to malt extract, again proved to be inviable. The parent culture was transferred to a new plate once again, in the normal course of maintaining the wild lines. On single sporing for the third time using this new colony, no indication of type D could be found, all the single spore lines being of type C. Type C had a growth rate indistinguishable from that of parent No. 11.

On examining previous data on No. 11 it was found that when first isolated its growth rate was only 3·25 mm. per 2 days, but that after 1 plate generation, *i.e.* two weeks, it had risen to 8·0 mm. per 2 days. The change could not be due to variations in the agar as all other lines remained constant in growth rate during this period. This suggests that No. 11 may have been similar in constitution to

certain synthetic heterocaryons described by Pontecorvo as being made from two homocaryons one with a wild type growth rate and the other carrying a biochemical mutation that was lethal on a minimal medium. He found that such heterocaryons were unlikely to have a growth rate higher than the wild homocaryon. This results in what Pontecorvo calls unbalanced heterocaryosis, in that a culture started from the heterocaryon resolves itself into the homocaryons, interspersed with a few strips of heterocaryon, that die out as they get further from the centre of inoculation. Obviously single hyphaeing from the edge of such a colony is more likely to yield the homocaryon with the wild type growth rate than anything else. This presumably has happened to our No. 11.

It is not easy to see why a heterocaryon from the wild should be unbalanced, but two possible explanations suggest themselves. First No. 11 was a single point infection of an exposed plate. If in the wild it had existed as a balanced heterocaryon of more than two component nuclei, it is possible that only two of these components might have been present in the cluster of conidia which were caught. A more plausible explanation, however, is that on the organic material, on which the parent of the spores which gave rise to No. 11 was presumably saprophytic in the wild, the heterocaryon was balanced. The lethal type of nucleus would presumably be able to grow normally if certain food requirements were satisfied, and so play its part in the heterocaryon. This is supported by the observation noted above that when No. 11 was first cultured on a minimal medium it grew very slowly, but that its growth soon increased to the rate found for homocaryon C. When first isolated the lethal type may have constituted a fair proportion of the nuclei of the heterocaryon. These would doubtless give different growth properties on the new, minimal, medium than they did on the natural substrate, so that the heterocaryon might well become unbalanced in its fresh circumstances. The lethal nucleus thus decreased in proportion, and as its alternative increased, the growth rate approached that of the homocaryon C, which was isolated from it. Eventually the lethal nucleus died out entirely and the heterocaryon was, in fact, converted into the homocaryon.

It appears, therefore, that No. 11 was a heterocaryon consisting of two genetically distinct types of nucleus, one of which carried a mutation that was lethal on a minimal medium, and doubtless similar in type to those produced artificially by X-rays, etc.

OTHER STRAINS

No. 4 has given a suggestion of being heterocaryotic in that it has saltated to give two types differing in growth rate, neither equal to the original isolate from the wild. Although other possibilities are open, it can be explained as the spontaneous breakdown of a two-phase heterocaryon. This possibility is being tested.

Only two of our collection, *viz.* Nos. 9 and 13, have proved to be homocaryons after careful examination.

DISCUSSION

The results described leave no doubt that genetical variation exists in wild *Penicillium*. They also show that heterocaryons are found in the wild and that these can have an advantage over homocaryons similar to that which hybridity confers on individuals of wild out-breeding sexually reproducing species.

An optimum growth rate associated with a certain ratio of the types of nucleus appears to exist for any particular medium. Thus the reconstituted heterocaryons and the original heterocaryon of No. 5 had the same growth rates, within experimental error, on the same medium. Furthermore, those investigated had the same nuclear ratios.

The nuclear ratios appear to be capable of alteration as a result of environmental changes. The change in growth rate of No. 11 together with the decrease in the number of lethal nuclei present in the heterocaryon, which was initiated after only one plate generation, suggests a rapid method of readjustment of a heterocaryon to such changes.

If heterocaryosis has such an adaptive significance in wild fungi, it would be expected that means would exist for the regular dispersal and propagation of the heterocaryons. It is of interest in this connection that, although the spores contain only one nucleus, colonies picked up as single point infections on exposed plates have given evidence of being heterocaryotic, *e.g.* No. 11 and No. 4. This suggests that the spores are held together during dispersal. They would thus maintain the heterocaryon in asexual reproduction on which this fungus now relies entirely, having abandoned the sexual cycle.

SUMMARY

Evidence has been obtained of the regular occurrence of heterocaryons in wild isolates of the *Asymmetrica* group of the genus *Penicillium*.

No. 5, isolated as a hyphal tip from a capsule of *Nicotiana rustica*, gave two types of colonies, A and B, on single sporing (the spores being uninucleate). A grew faster than B but not so fast as No. 5. The rates of germination of spores of No. 5, A and B and their growth on malt have also been investigated. No. 5, A and B are morphologically indistinguishable.

The heterocaryon was resynthesised by growing spores from homocaryons A and B together. It had a growth rate equal to No. 5, and, on single sporing, yielded types A and B in the same proportions as No. 5.

No. 11, obtained as a single point infection of an exposed plate, gave two types of single spore colonies, C and D. C had a growth

rate equal to that of the parent, while D gave only a microscopic growth on a minimal medium.

Only two isolates out of 16 have so far proved to be homocaryons.

This investigation was carried out while in receipt of a grant from the Agricultural Research Council. I am greatly indebted to Professor K. Mather, Director of the Unit of Biometrical Genetics, for his help and supervision both in the experiments and in the preparation of this paper. I should also like to express my thanks to Mr L. G. Wigan for his critical reading of the manuscript.

REFERENCES

BEADLE, G. W., AND COONRADT, V. L. 1944. Heterocaryosis in *Neurospora crassa*. *Genetics, 29*, 291.

HANSEN, H. N. 1938. The dual phenomenon in imperfect fungi. *Mycologia, 30*, 442.

PAPAZIAN, H. P. 1950. Physiology of the incompatability factors in *Schizophyllum commune*. *Bot. Gaz., 112*, 143.

PONTECORVO, G. 1946. Genetic systems based on heterocaryosis. *Cold Spr. Harb. Sym. quant. Biol., 11*, 193.

PONTECORVO, G., AND GEMMELL, A. R. 1944*a*. Genetic proof of heterocaryosis in *Penicillium notatum*. *Nature, 154*, 514.

PONTECORVO, G., AND GEMMELL, A. R. 1944*b*. Colonies of *Penicillium notatum* and other moulds as models for the study of population genetics. *Nature, 154*, 532.

SANSOME, E. 1946. Heterocaryosis, the mating type factors and sexual reproduction in *Neurospora*. *Bul. Torrey Bot. Cl., 73*, 397.

ASYNAPSIS AND SPONTANEOUS CHROMOSOME BREAKAGE IN SCILLA

HUBERT REES

John Innes Horticultural Institution and Department of Genetics, University of Birmingham

Received 27.i.51

1. INTRODUCTION

In 1928 Beadle and McClintock described a recessive Mendelian factor causing irregular meiosis in the form of a failure of pairing of homologous chromosomes at metaphase in *Zea mays*. At the time it was assumed that this abnormality was due to a failure of pachytene pairing, and the term asynapsis was used to describe it. Later Beadle (1933) reported that in " asynaptic " maize pachytene pairing was apparently normal, so that initial failure of pairing was not the cause of the later failure at metaphase. For convenience the term asynapsis was retained to describe the failure of metaphase pairing (*cf.* Koller on *Pisum*, 1938 ; and others) ; and it is in the same sense that the term is used in this account, implying that asynapsis may be due to effects becoming visible after a normal pachytene pairing as well as to failure of initial pachytene association.

Asynapsis has been described in a number or organisms, resulting from a variety of causes, *genotypic* and *environmental*. Among the most notable examples is that of " asynaptic " strains of *Crepis capillaris* (Richardson, 1935). Irregularities leading to non-pairing at metaphase were extensively reviewed by Darlington in 1937. In all these cases chiasma frequency shows a normal, unimodal variation.

In the following account asynapsis of a new kind is described in conjunction with spontaneous chromosome breakage. Such breakage in pollen mother cells would seem to be of rare incidence. A case mentioned in *Tulipa orphanidea* (Darlington and Upcott, 1940) was beyond analysis. Breakage may or may not have been associated with reduced chiasma frequency and consequent asynapsis.

2. MATERIAL AND METHOD

Bulbs of *Scilla sibirica* (variety Spring Beauty) from three sources were examined. The bulbs from two sources were triploid ($2n = 3x = 18$). Of seven bulbs examined from another source one was triploid, the other six diploid ($2n = 12$). In all six diploid plants spontaneous chromosome breakage was found in pollen mother cells. Evidently the " variety " consisted of two clones, one diploid and one triploid.

For anther squashes two methods proved satisfactory at the later stages of meiosis : (1) combined staining and fixing of fresh material using aceto orcein (La Cour, 1941), (2) fixation in three parts alcohol

TABLE 1

Distribution of cells lagging at prophase and of cells showing chromosome breakage at metaphase, in two bulbs

Bulbs	Prophase			Metaphase		
	Number of cells lagging	Normal cells	Per cent. lagging cells	Cells with chromosome breakage	Normal cells	Per cent. cells with breakage
1	69	896	7·1	52	845	5·8
2	24	1397	1·6	15	1589	0·94

and one part iron acetate in glacial acetic acid and staining in iron aceto carmine. For sectioning, anthers were fixed in La Cour's 2BE and stained by the Crystal Violet method.

3. FREQUENCY AND DISTRIBUTION OF ABNORMAL CELLS

The abnormalities are confined to pollen mother cells. The proportion of cells showing chromosome breakage varies from 0·94

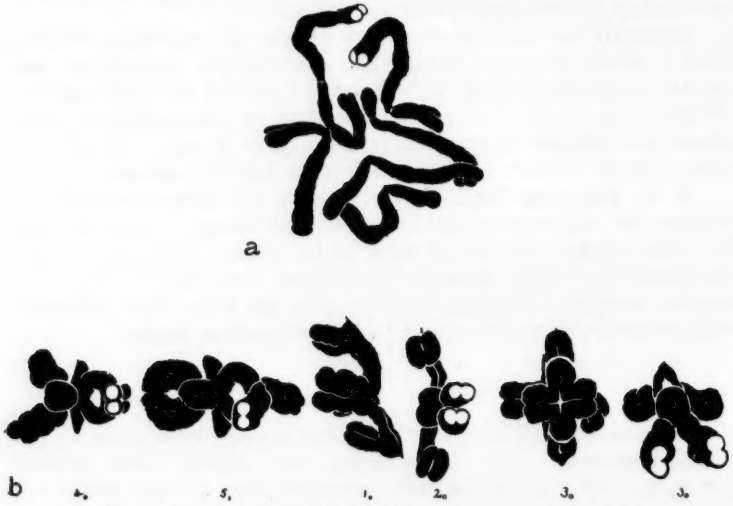


FIG. 1.—(*a*) Pollen grain mitosis in *Scilla sibirica*. (*b*) Normal bivalenta at metaphase.

per cent. in one plant to 5·8 per cent. in another. From examination of longitudinal sections of anthers the abnormal cells appear to be

localised only in so far as that none are seen towards the distal and proximal regions ; they are all scattered in the mid regions of the loculi.

4. MEIOSIS IN CELLS WITH NO CHROMOSOME BREAKAGE

Invariably six bivalents are found at metaphase (text fig. 1*b*), and meiosis follows a normal course. Bridges were seen at first anaphase in about 2 per cent. of cells, indicating inversion crossing over (plate, fig. 2). Of 22 cells with bridges counted out of 825 pollen mother cells at first anaphase in one plant, 5 cells show single bridges without fragments. In these cases the bridges may well be the result of failure of division of end genes (sister reunion), brought about by nucleic acid upset, as suggested by Darlington and Upcott (1940) in explanation of similar bridges seen in *Tradescantia* (Darlington, 1937), and in *Pisum* (Koller, 1938), and also at mitosis in *Trillium* (Darlington and La Cour, 1945).

Pollen grains appear normal and divide regularly (text fig. 1*a*).

5. MEIOSIS IN CELLS WITH CHROMOSOME BREAKAGE

The breakage of chromosomes becomes evident generally at metaphase except in a few cells where highly fragmented nuclei occur which seem incapable of organising a recognisable metaphase. The degree of breakage is extreme and accurate analysis of breakage and reunion impossible in all but a few cells (text figs. 3*a* and 3*b*). Difficulty of anaphase separation (see text fig. 2*b*) may be a consequence of obstructed reproduction of chromosomes, as in *Trillium* (Darlington and La Cour, 1945). Chromosome contraction is semi mitotic.

There is no evidence of chiasma formation ; hence there are no bivalents, and the first division is mitotic. All the univalents or centric " univalent " fragments divide at the first division in the great majority of cells (text figs. 3*a* and 3*b*). During this division the acentric fragments fall apart. Only in one cell (text fig. 2*c*) was there any sign of a second division. The cells, containing a number of micronuclei, generally degenerate after one division.

Apart from the disorganisation brought about by fragmentation, the fact that all the univalents divide at the first division may in itself preclude a second division, for in consequence, the interphase centromeres and gene strings are " single," in contrast to their potentially and actually " double " state at normal meiotic interphase. Univalents divide once only (Darlington, 1939), and if all divide together as in this instance the " single " condition of interphase centromeres is probably effective in *not* initiating a second division. In the one exception it is reasonable to assume that not all the centric pieces had divided at first anaphase.

Text figs. 3*a* and 3*b* show homologous univalents in close association at anaphase, which leads to the assumption that asynapsis

is not brought about by failure of pachytene pairing, but by failure of chiasma formation between paired homologues. This was found to be the case in " asynaptic " *Crepis* (Richardson, 1935), in *Pisum* (Koller, 1938) and in *Lilium-testaceum* (Ribbands, 1937). Unfortunately preparations of early stages of meiosis in this material were not good enough to verify this assumption cytologically.

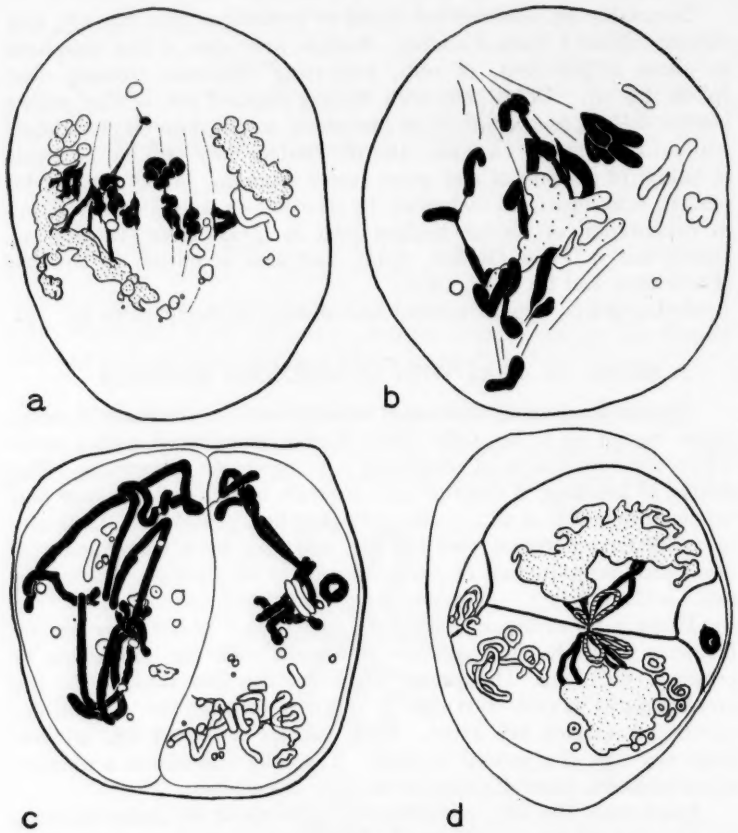


FIG. 2.—(*a*) First anaphase in an abnormal cell. Chromatid bridges are the result of sister reunions following chromosome breakage. (*b*) First anaphase showing bent spindle. At 9 o'clock failure of separation presumably arises through failure of reproduction of the chromosome. (*c*) Second division. (*d*) First telophase. Chromatid bridges are trapped by the cell wall formed. Centric fragments are shown in black. ×960.

Spindles are frequently bent and stretched (text fig. 2*b*), a feature noticed in many cases of asynapsis (Dobzhansky in *Drosophila*, 1934 ; Brieger in *Nicotiana*, 1934).

6. KIND OF CHROMOSOME BREAKAGE

Metaphase and anaphase configurations point to chromosome breaks (B^{11}), followed sometimes by reunion, giving rise to, (1) chromatid bridges at anaphase (text figs. 2*a*, 2*b*, 3*a* and 3*b*), (2) monocentric rings (text figs. 2*c* and 2*d*), (3) dicentric rings (text fig. 3), (4) acentric fragments which often form rods or rings by reunion. The consequences of breakage and reunion are in fact, from the point of resultant chromosome configurations, similar to those induced artificially by X-rays, etc.

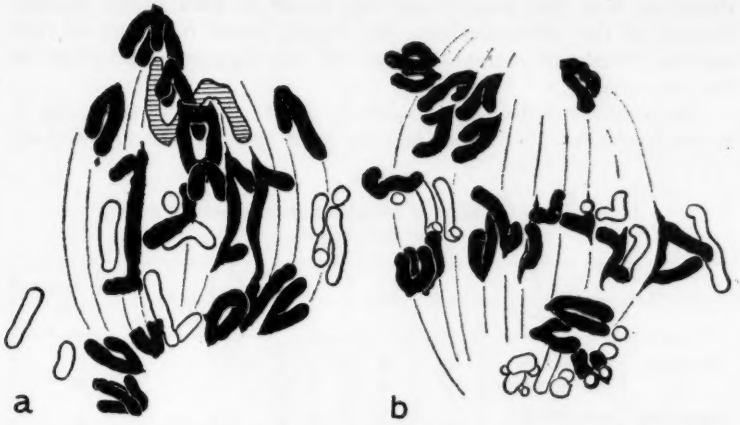


FIG. 3.—(*a*) First anaphase. Homologous univalents at 12 o'clock and 7 o'clock are closely associated. The cross hatched structure is interpreted as being a displaced centric fragment which has undergone sister reunion. Two dicentric chromatid rings have separated towards each pole. Three chromatid bridges appear on the equator. (*b*) First anaphase. Paired homologous univalents appear at each pole, chromatid bridges at the equator. No bivalents appear in either case. In both cells the centric pieces are drawn in black. ×960.

There is evidence for fragmentation resulting from severing, by cell wall formation of chromatid loops (text fig. 2*d*), the loops probably being a consequence of " slack " chromatid bridges, bridges such as may arise by sister reunion at a distance from the centromere greater than half the length of the spindle axis. In another case (plate, fig 3) part of the chromosome complement of a cell showing much breakage appears trapped by the cell wall between itself and an adjacent normal cell, so that some parts of the chromosomes are contained in the " normal " partner. This in itself would no doubt result in fragmentation, but as this is not a constant feature associated with abnormal cells it cannot be an important contributing cause of breakage. It does however suggest that irregularity in some cases arises at pre-meiotic mitosis.

7. TIMING ANOMALIES

At early prophase a number of cells lag (plate, fig. 1). Thus when the majority of cells are at early diplotene a minority are at leptotene. The frequency of these laggard cells corresponds with the frequency of cells which show breakage at metaphase in anthers of other flowers of the same plant (table 1).

It appears therefore that a timing difference at early stages is associated with the breakage evident at later stages. It was mentioned earlier that some cells by virtue of very great fragmentation seem incapable of forming a metaphase plate. These cells probably often disappear from the record, and this would at least partly explain, in each of the above analyses, the slightly lower frequency of cells showing breaks at metaphase than of cells lagging at prophase in the same plant.

Metaphase in affected and normal cells, as we see in plate, fig. 2, is synchronous. The initial lagging must therefore be followed by

TABLE 2

A comparison of diameters of normal and abnormal cells at prophase and at first metaphase

Diameters in μ		*35-40*	*40-45*	*45-50*	*50-55*	*55-60*	Mean diameter in μ
Prophase .	normal cells .	6	8	1	...	...	41
	lagging cells .	...	4	6	3	2	49
Metaphase	normal cells .	2	5	8	...	...	45
	"broken" cells	...	...	2	6	7	54

The probability of obtaining by chance such differences in diameter between the two classes of cells at prophase and at metaphase is less than 0·001. The results were tested by the *t* method.

an acceleration of the division process. Such an acceleration of retarded cells has been inferred by Darlington and La Cour from observations of cells X-rayed to produce chromosome breakage (1945).

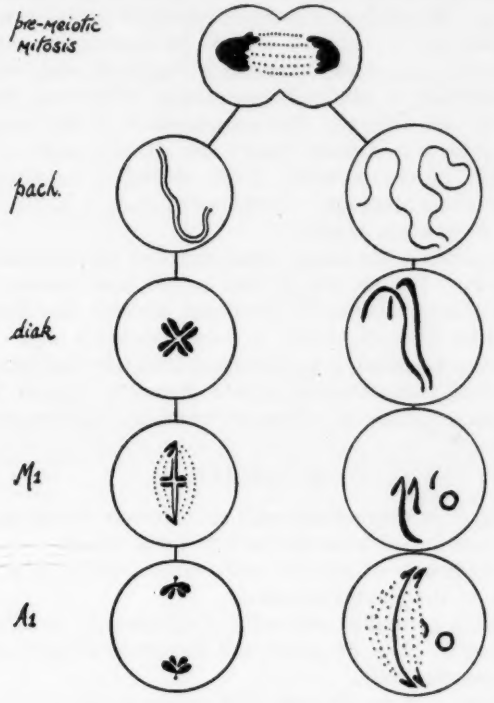
8. CELL SIZE DIFFERENCE

Table 2 shows that a significant difference exists between cell diameters of normal and abnormal cells at prophase and at metaphase. As these measurements were made from squash preparations an error accentuating the difference may have been introduced, but nevertheless that a significant difference does occur is beyond doubt (plate, figs. 1 and 2).

A somewhat similar correlation between size difference and difference in behaviour was found by Beadle (1933), where failure of cell wall formation following the first meiotic division was a characteristic of "giant" pollen mother cells in maize.

9. CAUSE AND EFFECT

The increased volume suggests the possibility that a differentiation in the irregular cells has led to a prolonged growth phase, that is to protein synthesis at the expense of nucleic acid synthesis. Later, the onset of division in these sick cells may be due to stimulation by the



Abnormality resulting from differentiation of sister cells following pre-meiotic mitosis.

Diagram.

surrounding actively dividing cells. There would in consequence be a reduced precocity of meiosis in the irregular cells, and failure of chiasma formation is probably due to a reduced precocity of meiosis (Darlington, 1937).

One can visualise on this basis a sequence of events similar to that suggested to occur in " asynaptic " maize by Beadle (1933), that is

a precocious splitting at pachytene with consequent failure of chiasma formation between the paired chromosomes. In maize, however, asynapsis was not complete.

The property of the plant to show abnormality in its pollen mother cells is interpreted as being genetically controlled. The threshold of expression of the irregularity must be a delicate one, even to the point of differentiation between sister cells at pre-meiotic mitosis (plate, fig. 3). An analogous sharp threshold for breakage was inferred by Darlington and Upcott (1940), and by Darlington and La Cour (1945). Such a controlled change, it is suggested, may well take the form of a reduction in cell wall permeability which may lead to gross differences in cell activity; the consequences of this being first, to prolong growth and thereby retard the division cycle of the cell, and, secondly, by the products of this altered metabolism, to bring about chromosome breakage. Abnormality may, as has been inferred, arise at the pre-meiotic mitosis.

That asynapsis is the direct consequence of chromosome breakage seems unlikely. Mather (1934) and others have shown that fragmentation induced artificially does not prevent the formation of chiasmata, even when the degree of fragmentation is high. Asynapsis in *Scilla sibirica* is complete in abnormal cells, chromosome breakage variable. Both abnormalities would therefore appear to be independent consequences of a common metabolic disturbance.

10. SUMMARY

1. Asynapsis in conjunction with spontaneous chromosome breakage is described in pollen mother cells of *Scilla sibirica*.

2. The frequency of affected cells varies from 0·94 per cent. to 5·8 per cent. in the plants examined.

3. Meiosis is normal in cells without chromosome breakage.

4. Lagging at early prophase and increased volume are characteristic of abnormal cells.

5. Breakage is of the B^{11} type, with results similar to those brought about artificially by X-rays, etc.

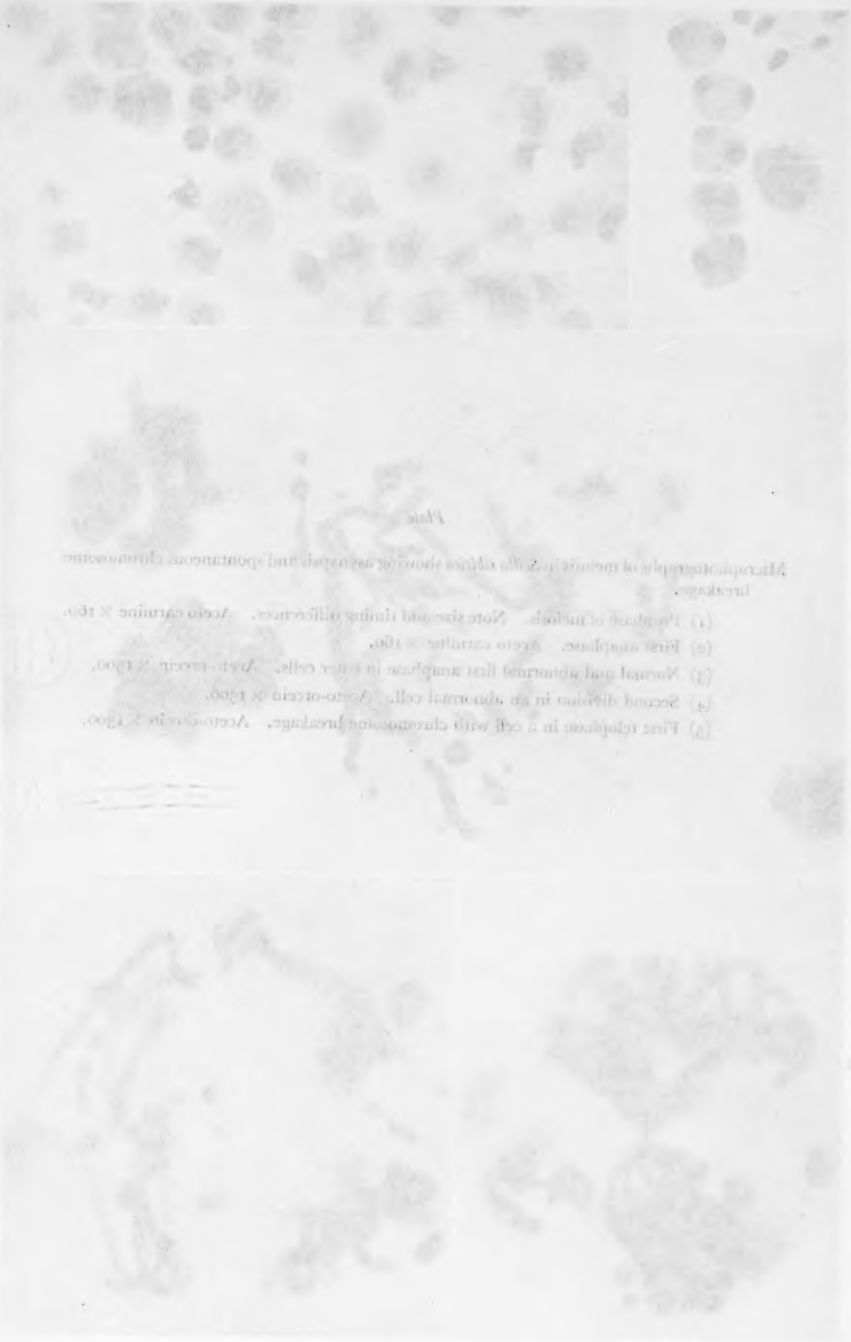
6. Asynapsis and chromosome breakage are interpreted as being independent consequences of a common cell disturbance with a sharp threshold, differentiating genetically equivalent premeiotic cells.

I am pleased to thank Dr Darlington and Mr La Cour for their advice and criticism, and for providing facilities which enabled me to carry out this work at the John Innes Horticultural Institution.

11. REFERENCES

BEADLE, G. W., AND MCCLINTOCK, B. 1928. A genic disturbance of meiosis in *Zea mays*. *Science*, *68*, 433.

BEADLE, G. W. 1932. A gene in *Zea mays* for failure of cytokinesis during meiosis. *Cytologia*, *3*, 142-155.



Plate

Microphotographs of meiosis in *Scilla sibirica* showing asynapsis and spontaneous chromosome breakage.

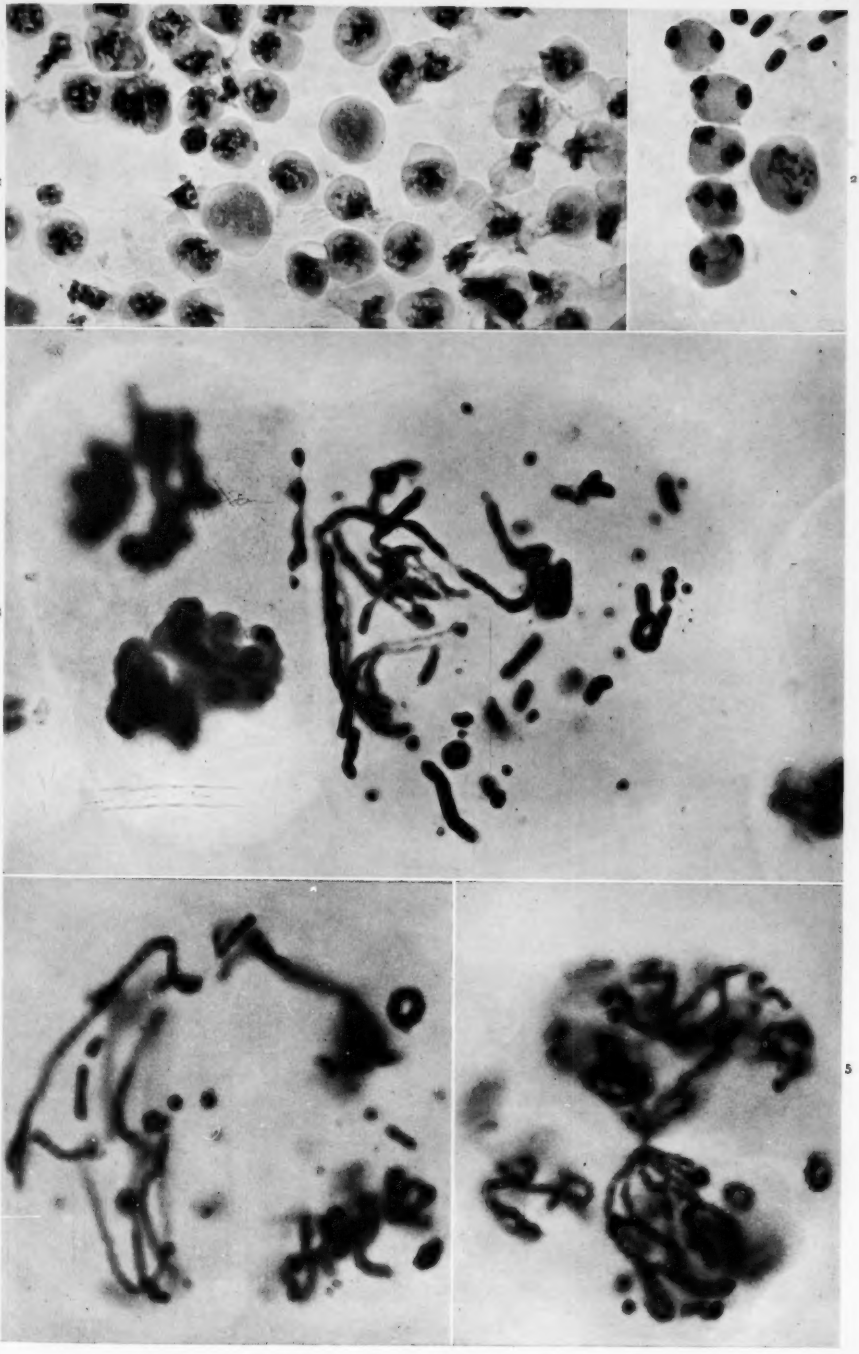
(1) Prophase of meiosis. Note size and timing differences. Aceto carmine × 160.

(2) First anaphase. Aceto carmine × 160.

(3) Normal and abnormal first anaphase in sister cells. Aceto-orcein × 1300.

(4) Second division in an abnormal cell. Aceto-orcein × 1300.

(5) First telophase in a cell with chromosome breakage. Aceto-orcein × 1300.



BEADLE, G. W. 1933. Further studies in asynaptic maize. *Cytologia*, *4*, 269-287.

BRIEGER, F. 1934. Ablenf der Meiose bei volliges Asyndese. *Ber. Deuts. Bot. Ges.*, *52*, 149-153.

DARLINGTON, C. D. 1937. *Recent Advances in Cytology*. London : Churchill.

DARLINGTON, C. D. 1939. Misdivision and genetics of the centromere. *J. Genet.*, *37*, 341-364.

DARLINGTON, C. D., AND UPCOTT, M. B. 1940. Spontaneous chromosome change. *J. Genet.*, *41*, 297-338.

DARLINGTON, C. D., AND LA COUR, L. F. 1945. Chromosome breakage and the nucleic acid cycle. *J. Genet.*, *46*, 180-267.

DOBZHANSKY, TH. 1934. Studies on hybrid sterility. I. Spermatogenesis in pure and hybrid *Drosophila pseudo-obscura*. *Zeitz. Zellf. u. mikr. Anat.*, *21*, 169-223.

MATHER, K. 1934. The behaviour of meiotic chromosomes after X-irradiation. *Hereditas*, *20*, 303-322.

RIBBANDS, C. R. 1937. The consequencies of structural hybridity at meiosis in *Lilium testaceum*. *J. Genet.*, *35*, 1-24.

RICHARDSON, M. M. 1935. Meiosis in *Crepis* I and II. *J. Genet.*, *31*, 101-117, 119-143.

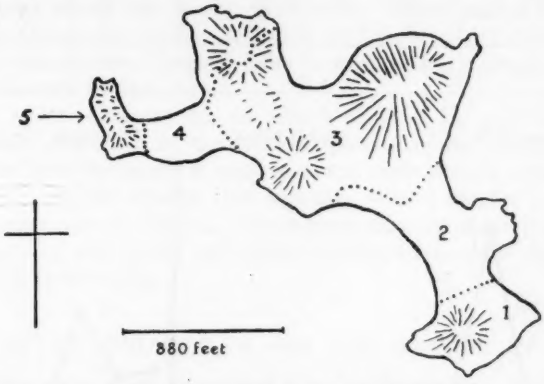
THE DISTRIBUTION OF SPOT-NUMBERS AS AN INDEX OF GEOGRAPHICAL VARIATION IN THE BUTTERFLY *MANIOLA JURTINA* L. (LEPIDOPTERA : SATYRIDAE)

W. H. DOWDESWELL
Science School, Winchester College
and
E. B. FORD
Oxford, England

Received 4.iv.51

I. PREVIOUS WORK AND TECHNIQUE

DOWDESWELL, FISHER AND FORD (1949) have already shown that a striking sexual difference exists between the distribution of spotting on the underside of the hind-wings of *Maniola jurtina* (the Meadow Brown butterfly). For purposes of comparison, these previous data must briefly be summarised and restated.



MAP.—Tean (Isles of Scilly). Boundaries of collecting areas . . . . . .

They were obtained in August 1946 on the uninhabited island of Tean, Isles of Scilly, by Dowdeswell and Ford, who were using this species for certain quantitative studies. The island can be divided into five areas, representing two main types of ecological habitat. Areas 1, 3 and 5 are regions of rocky or rough ground covered with a thick vegetation of bracken, bramble, gorse and long grass, and they are inhabited by *M. jurtina*. They are separated from one another by areas 2 and 4, which take the form of narrow necks of land. These are sandy, lawn-like, clothed in close short turf and low plants such as *Armeria maritima*, and *M. jurtina* is absent from them.

Area 2 is approximately 220 yards long and separates a rocky promontory (area 1) from the main central part of the island (area 3). This latter is similarly separated from a smaller rocky promontory (area 5) by area 4, which is about 150 yards long. The island is roughly crescentic in form, and nearly 1000 yards long by 400 yards wide at its greatest dimensions (see map).

TABLE 1

Numbers of spots per hind wing of Maniola jurtina *from three colonies on Tean, 1946*

Spot numbers	Male				Female			
	Areas			Totals	Areas			Totals
	I	III	V		I	III	V	
0	10	11	2	**23**	63	82	25	**170**
1	34	47	6	**87**	52	73	29	**154**
2	115	203	21	**339**	92	145	39	**276**
3	41	45	7	**93**	34	55	12	**101**
4	11	12	2	**25**	10	10	4	**24**
5	...	2	...	**2**	3	1	...	**4**
Totals .	211	320	38	**569**	254	366	109	**729**

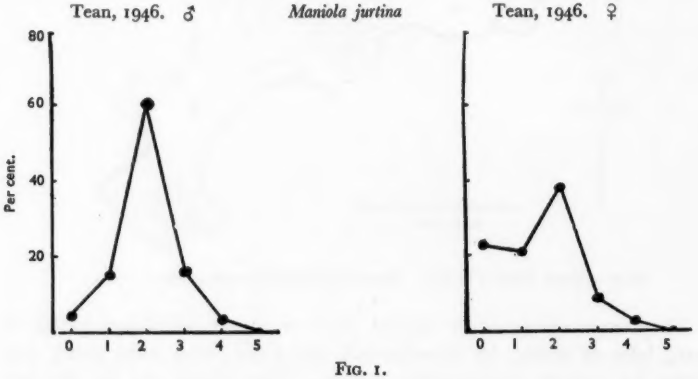


FIG. 1.

By means of marking, release and recapture we had shown that in 1946 the whole population of *Maniola jurtina*, a single-brooded species, somewhat exceeded 3000 in area 1, that it exceeded 15,000 in area 3, and that it was less than 500 in area 5. We also demonstrated that areas 2 and 4 constitute remarkably effective barriers between the three areas inhabited by *M. jurtina*; the leakage across from one to another being quite small.

As we were anxious to test whether this isolation had caused any divergence in the three populations, we recorded the number of spots on the under side of the hind-wings. We chose this character because it could be studied on the living insect, a necessity in view of the other work on which we were engaged, and because it was likely to be multifactorial : a type of variation which responds quickly to the effects of selection.

Our scoring technique must briefly be explained. The spots are situated in definite positions on the under side of the hind-wings, and they vary in number from 0 to 5. Since they proved to be symmetrical on the two wings, with rare exceptions (which we recorded), we arbitrarily decided to score the left side. The spots of course vary in size, but it would have been impossible to quantify them ; indeed to record the spots as present or absent is a valid procedure if it provides consistent results. In some regions (*e.g.* the Isles of Scilly) the wings are powdered with blackish scales, and occasional black scales occur on the grey or brown hind-wings in all races. A spot was regarded as absent if it could not be distinguished from a black scale which might have occurred anywhere on the wing. We scored the spots independently of one another, and discussed those about which any doubt could arise. When one of us was at work examining and scoring the insects, he did not see his accumulating frequency-distribution (recorded by the other) lest unconscious bias might influence his decisions.

Our results obtained on the island of Tean in 1946 indicate a remarkable difference in the type of spotting of the two sexes : in the males the distribution is approximately symmetrical, with a mode at 2 spots ; in the females it is bimodal, with a smaller mode at 0 and a larger one at 2 spots. We showed that the three populations (in areas 1, 3 and 5) did not differ significantly in their distribution of spotting (table 1, fig. 1).

2. RESULTS OBTAINED IN 1950, AND ANALYSIS OF DATA

Further study made us suspect that the distribution of spotting in *M. jurtina* constitutes a more complex and remarkable problem than we had at first supposed. We therefore took up this subject again in 1950.

Manifestly it was impossible to make comparisons between Tean in 1946 and other areas four years later, since the relative frequency of spotting may vary from year to year. We therefore obtained a further set of results for all three areas on the island of Tean in August 1950. In the males, the frequencies were very similar to those in 1946 ; in the females they retained their general type (bimodal, with a lesser mode at 0 and a greater at 2 spots) but differed in degree, the bimodality being accentuated (table 2, fig. 2). The situation in this sex therefore requires some analysis.

The heterogeneity of female spotting for the three populations was determined from a table of general contingency. In order to obtain observations for each comparison, the frequencies for 4 and

TABLE 2

Spot-numbers per hind wing of Maniola jurtina *from three colonies on Tean, 1950*

Spot numbers	Male				Female			
	Areas			Totals	Areas			Totals
	I	III	V		I	III	V	
0	2	2	2	**6**	39	45	48	**132**
1	9	7	4	**20**	23	21	22	**66**
2	58	38	27	**123**	40	59	71	**170**
3	27	7	6	**40**	9	18	18	**45**
4	8	3	2	**13**	6	4	5	**15**
5	3	2	...	**5**	1	...	3	**4**
Totals .	107	59	41	**207**	118	147	167	**432**

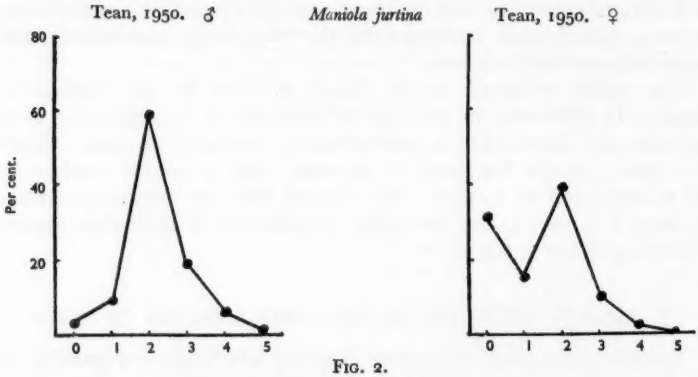


FIG. 2.

5 spots were added together (since the entry for 5 spots for area 3 is blank). We thus have 3 columns (areas) and 5 rows (spots), giving 8 degrees of freedom, and the χ^2 for the heterogeneity of these data is 6·8, for which P lies between 0·7 and 0·5. Thus on the basis of spot-numbers, the three populations on Tean in 1950 are again substantially identical.

The difference between female spotting on Tean in 1946 and 1950 may be judged significant. It is obtained from a $2 \times n$ table as a χ^2 of 13·8 for 5 degrees of freedom, there being 2 columns (the two years), 6 rows (0 to 5 spots), and no missing terms ; for which P is just under ·02.

In 1950, with the help of several collaborators, we made collections of about 50 males and 50 females (in two instances 100 of each sex) from one locality each in a transect of eight counties across Southern England : S.W. Cornwall, mid-Devon (Tiverton), central Somerset (Taunton), Wiltshire (Salisbury), Oxford, Warwickshire (Rugby), Kent (Canterbury), and S.E. Suffolk (Ipswich).

The Cornish sample must be considered separately. Those from the seven remaining counties present certain curious features. First, the distribution of spotting in the males is practically identical throughout and with that on Tean. Secondly, the spotting of the females is entirely unlike that of the males or of the Tean females, being unimodal and descending steeply from a high value at 0 spots (table 3, fig. 3).

TABLE 3

Spot-numbers per hind wing of Maniola jurtina *from seven English counties*

Spot numbers	Male							
	Devon	Somerset	Wilts	Oxon	Warwick	Kent	Suffolk	Totals
0	...	...	1	1	3	1	3	9
1	8	3	6	7	8	4	15	51
2	54	45	39	36	31	32	61	298
3	8	4	13	4	7	11	20	67
4	2	1	2	1	...	1	2	9
5	...	...	1	...	...	1	1	3
Totals .	72	53	62	49	49	50	102	437
	Female							
0	37	18	26	37	26	24	63	231
1	15	6	8	12	10	11	21	83
2	12	3	8	4	6	10	17	60
3	3	1	1	1	1	4	4	15
4	...	...	...	...	2	1	...	3
5	...	...	...	...	...	...	1	1
Totals .	67	28	43	54	45	50	106	393

It is first necessary to consider whether the samples from these seven counties, from Devon to East Suffolk, are homogeneous and can be combined to give a grand total. This is determined from a general contingency table of 7 columns (the counties) and, potentially, of 6 rows (the spots). However, it will be seen from table 3 that there are numerous missing entries in the last two rows, so that the distributions from 3 to 5 spots were accumulated. It was then evident that χ^2 for the majority of the terms in the lowest (combined) row was quite small (generally much under 5). It was therefore thought proper to accumulate one row more, giving a table of 7

columns and 3 rows, so having 12 degrees of freedom. The heterogeneity χ^2 is 11·79, and none of the contributing values is inconsistently large. For this, P is approximately 0·5. Thus we have clear evidence that the female spotting is substantially equivalent in these seven counties.

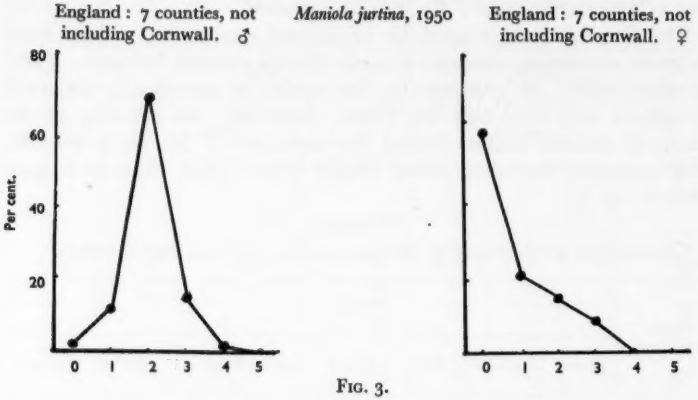


FIG. 3.

The difference between female spotting on Tean (1950) and for the seven English counties under consideration is obviously so great that it is unnecessary to measure it accurately. Indeed a single term of the $2 \times n$ table concerned (2 columns and 6 rows), that for 2 spots, supplies a χ^2 of 42·8. There is no need to go further.

The result from S.W. Cornwall (St Feock, near Falmouth) is of a remarkable kind, being quite distinct from that obtained from the rest of England, as indicated by our highly consistent sample of seven counties. The male frequencies are again approximately identical with all others, but those of the females are almost perfectly intermediate between the rest of England and Tean (table 4, fig. 4). For

TABLE 4

Spot-numbers per hind wing of Maniola jurtina *from*

Spot numbers	S.W. CORNWALL		ST MARTINS		TRESCO	
	Male	Female	Male	Female	Male	Female
0	1	39	5	33	2	43
1	5	24	16	35	7	37
2	68	29	66	32	55	41
3	16	7	20	7	15	7
4	7	...	7	2	4	2
5	3	...	3	...	2	...
Totals .	100	99	117	109	85	130

female spotting in S.W. Cornwall is bimodal, as on Tean, but the mode at no spots is the greater. As explained, this is the modal value in the rest of England, while 2 is the larger mode on Tean.

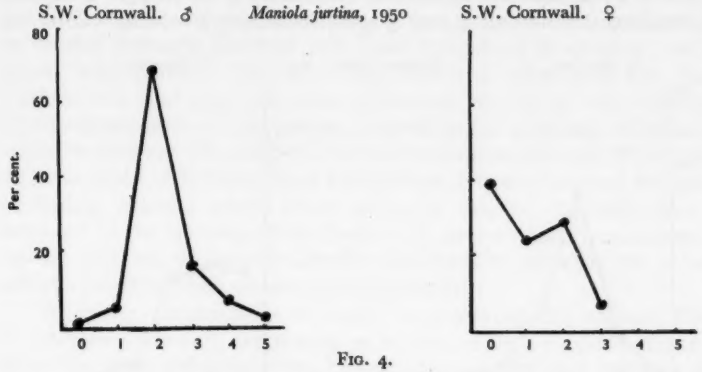


FIG. 4.

The difference between the rest of the combined English samples and the Cornish one is clearly significant. It is obtained from a $2 \times n$ table of 2 columns and 4 rows (combining 3 to 5 spots to escape missing values). This gives 3 degrees of freedom for which $\chi^2 = 15{\cdot}1$, so that P is substantially less than ·01.

The difference between female spotting in S.W. Cornwall and Tean (1950) is also significant. $\chi^2 = 11{\cdot}6$ for 3 degrees of freedom, combining three or more spots to avoid missing values. For this, P is approximately ·01.

We also obtained samples on two other islands in Scilly, Tresco (table 4, fig. 5) and St Martins (table 4, fig. 6). Once more, the male

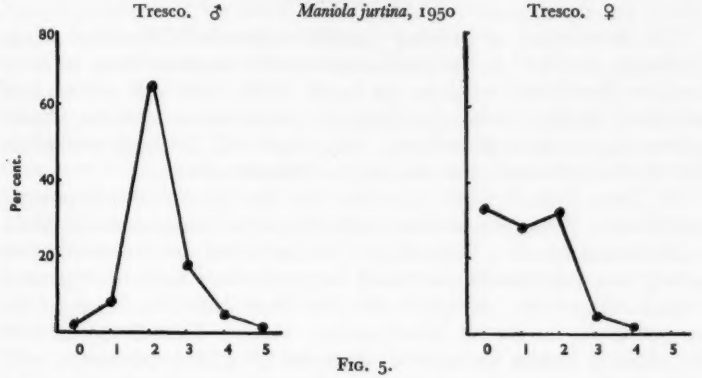


FIG. 5.

frequencies are practically the same as in all the other areas. It is remarkable, however, that the distribution of female spotting on these

two islands is approximately identical ($\chi^2 = 0{\cdot}7$ for 4 degrees of freedom, for which P nearly reaches 0·95), while each differs significantly from the female spotting on Tean (1950). The difference between Tean and St Martins is measured by $\chi^2 = 18{\cdot}4$ for 4 degrees of freedom (combining 4 and 5 spots to escape a missing value), so

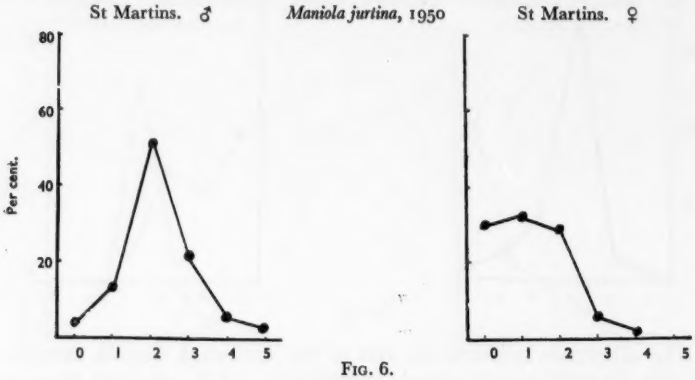


FIG. 6.

that P is approximately ·001. Similarly, the values for the comparison between Tean and Tresco are $\chi^2 = 16{\cdot}2$ for 4 degrees of freedom, so that P_2 approaches ·001.

The approximately flat-topped distributions of female spotting on St Martins and Tresco do not differ significantly from the Cornish sample. For Cornwall compared with St Martins we have $\chi^2_{(3)} = 2{\cdot}4$, with P approximately 0·5. For Cornwall compared with Tresco $\chi^2_{(3)} = 1{\cdot}1$, for which P lies between 0·8 and 0·7.

3. DISCUSSION

The distribution of spotting on the underside of the hind wings on *Maniola jurtina* is in the males uniformally constant in a number of widely distributed areas in the south of England and on three of the Isles of Scilly. It is approximately symmetrical. In the female it never approaches that form, being not only without exception different from the male but varying in different areas.

On Tean, Isles of Scilly, it is clear that the distribution of spotting is stabilised. It has already been shown that the three areas inhabited by *M. jurtina* are to a high degree isolated from one another. Yet spotting was substantially identical in all of them both in 1946 and in 1950. Moreover, though in the female it differs as between the two years, to an extent which about reaches formal significance (P = ·02), it retains its general character as a bimodal curve, with the mode at no spots the smaller and that at 2 spots the greater.

Turning now to the seven counties of England from Devon to South-east Suffolk, we again have stability, not only in the males

which we have not yet found to vary, but in the females in which the distribution of spot numbers is quite distinct from that on Tean. It should be noticed that the localities in these seven counties were chosen to supply the maximum of available information. In Britain the isotherms run approximately north and south, while the difference in rainfall between Tiverton and Ipswich is about as great as can be found in England. That is to say, Devon is warm and wet, East Suffolk cold and dry, yet these differences are in no way reflected in the population of *M. jurtina*, judged by its spotting. Similarly, when we compare the samples from the neutral to acid soil of Tiverton and the chalk of Salisbury and Canterbury, bearing in mind the great ecological changes which these soil-types involve, no difference is reflected in the spotting of the female *M. jurtina* which, variable as it clearly can be, is again evidently stabilised by selection in a way which over-rides such environmental diversity.

We were particularly fortunate in receiving the sample from South-west Cornwall, suggesting, as it does, a cline : one that passes from the form stabilised from Devon across to the east coast to the type of female spotting found on Tean, Isles of Scilly. It will be of great interest to investigate this matter further, which we intend to do. Clearly some material change occurs between Devon and South-west Cornwall. It will be necessary to find if it is gradual or relatively abrupt and, if the latter, with what physical features (if any) it can be correlated.

The race of *Maniola jurtina* inhabiting Scilly is regarded as a subspecies (*cassiteridum* Graves). It is distinguished by its colour and pattern. The fact that the form from South-west Cornwall is in spotting approximately intermediate between that on Tean and the rest of England, and that this Cornish form differs so little from that on the islands of St Martins and Tresco, does not suggest that the subspeciation, if it be valid, contributes any sharp distinction to the character now under consideration.

The situation so far revealed in the Isles of Scilly is remarkable and merits detailed study. We have evidence (cited) that female spotting is stabilised on Tean. Also that it is stabilised on the islands of St Martins and Tresco, for it is nearly identical on them. Yet these three islands lie roughly on the arc of a wide circle *with Tean between the other two.*

St Martins and Tean approach to within 300 yards at one point, though from there the coasts fall rapidly away. They have, however, been separated much longer than have most of the Isles of Scilly for, unlike the condition found elsewhere, the channel between them is deep : it probably represents the course of the stream which drained the " roadstead " of Scilly when this was a low plain. Dowdeswell, Fisher and Ford (1949) showed that minor ecological barriers present powerful obstacles to the movement of *M. jurtina* even on land, and that there is little or no interchange between Tean and St Martins

(as indeed the spot-distributions also suggest). Tresco is further away, being about three-quarters of a mile from Tean as its nearest point.

We have at present no means of interpreting the close similarity of *M. jurtina* on St Martins and Tresco and its striking difference on Tean which lies between them. St Martins and Tresco are relatively large islands (about 2 and 2¼ miles long respectively) compared with Tean, the length of which is just under 1000 yards. Taking into account the relative shapes of these three islands, the area of Tean must be rather less than 1/12 of that of St Martins and smaller still compared with Tresco. Separation into small isolated or semi-isolated populations is certainly favourable to rapid evolutionary progress, not through genetic " drift " as Sewall Wright has suggested but because of the variety of environmental conditions to which the various colonies are individually exposed (Fisher and Ford, 1947; Sheppard, 1951). Thus it may be that divergence has been pushed further upon the small islands, each presenting special characteristics of habitat, than upon the larger ones where the populations, numerically far greater, meet a wider set of conditions on the average much more alike from one island to another. The opportunities for rapid evolutionary adjustment on the smaller islands will be assisted by fluctuations in numbers from one season to another (which we have detected and measured on Tean). The larger and smaller islands of Scilly must be examined from this point of view.

Finally we propose to investigate the genetics of spotting in *M. jurtina*. It is clear that the situation here outlined opens up a number of possibilities for the study of evolution in the field.

4. SUMMARY

1. The number of spots on the underside of the hind-wings of the butterfly *Maniola jurtina* proves to be a useful index of evolutionary divergence in that species.

2. The distribution of spotting is entirely dissimilar in the two sexes. So far as our investigations go, it is approximately symmetrical and everywhere similar in the male. In the female, however, its frequencies are always different from those in the male and they may vary from one locality to another.

3. On Tean, Isles of Scilly, female spotting is bimodal (a smaller mode at 0 and a larger at 2 spots). It is shown that this arrangement is subject to considerable stability.

4. The frequency of spotting is unimodal (a large mode at 0) in seven localities scattered across the south and midlands of England from mid-Devon to East Suffolk. Very considerable environmental differences of temperature, humidity, and soil characterise these places, yet the spotting is of uniform type throughout.

5. A sample from south-west Cornwall suggests a cline between the form found in the rest of England and that on Tean, Isles of Scilly.

6. Female spotting is practically identical on two larger islands in Scilly, St Martins and Tresco, though these lie on either side of Tean where it is quite distinct from them.

7. The opportunities which these data supply for a study of evolution in the field are briefly discussed.

Acknowledgments.—We wish to express our grateful thanks to Major A. A. Dorrien-Smith for his kind permission to camp on Tean. Also to those who were so good as to collect samples of *M. jurtina* for us : Mr S. Beaufoy at Ipswich, Mr L. P. and Mr M. F. ffrench-Constant in south-west Cornwall, Mr R. R. Menneer at Salisbury, and Members of the following School Natural History Societies : Blundell's, Rugby, St Edmund's School, Canterbury, and Taunton School.

5. REFERENCE

DOWDESWELL, W. H., FISHER, R. A., AND FORD, E. B. 1949. *Heredity*, *3*, 67-84.
FISHER, R. A. AND FORD, E. B. 1947. *Heredity*, *1*, 143-174.
SHEPPARD, P. M. 1951. *Heredity*, *5*, 349-378.

THE INHERITANCE OF THE *DUFFY* BLOOD GROUPS: AN ANALYSIS OF 110 ENGLISH FAMILIES

R. R. RACE and RUTH SANGER

Medical Research Council Blood Group Research Unit, Lister Institute of Preventive Medicine, London, S.W. 1

Received 7.ix.51

THE *Duffy* blood group system was discovered by Cutbush, Mollison and Parkin in 1950. The anti-*Duffy* serum, anti-Fy^a, divides human beings into two phenotypes, those whose red cells are sensitised, $Fy(a+)$, and those whose red cells are not, $Fy(a-)$.

Cutbush and Mollison (1950) tested 27 families with 49 children and demonstrated that the antigen Fy^a is inherited by means of a gene which expresses itself in single as well as in double dose. This was confirmed by Race, Holt and Thompson (1951) who tested 58 families with 148 children.

Anti-Fy^a has not yet been shown to make a dosage distinction between bloods representing the genotype Fy^aFy^a and those representing the genotype Fy^aFy^b; nor has an antiserum anti-Fy^b yet been identified. Fy^a is therefore in the phase, usually fleeting for blood group antigens, in which it may properly be called a dominant Mendelian character.*

The present paper reports the results of testing a further 52 families for the *Duffy* groups. These families are shown in table 1, together with the 58 families of Race, Holt and Thompson; the total of 110 families with 274 children are analysed below.

Ascertainment.—The families of table 1 were quite unselected as far as blood groups are concerned. There was some selection for families with two or more children.

Twins.—When a pair of twins was not shown to be dizygous by sex or by any of the blood groups it has been scored as one child.

Other blood group systems.—The families have also been tested for the A_1 A_2 *B O, MNS, P, Rh, Lutheran, Kell* and *Lewis* groups. About half of them have also been tested for the *Kidd* groups.

Linkage.—In the previous paper (Race *et al.*, 1951) it was reported that no evidence could be found for linkage between the *Duffy* genes and the genes for other blood groups, or for phenyl thio-carbamide testing; nor was there any evidence for partial sex linkage of the *Duffy* genes. The present addition of 52 families supports this independent segregation of the *Duffy* genes. Linkage calculations are not given for they form part of a paper on linkage and the blood groups at present in preparation (Holt, Thompson, Sanger and Race).

* Since the writing of this paper both an anti-Fy^a showing a dosage effect and an example of anti-Fy^b have been found.

GENE FREQUENCIES

The gene frequencies used in the calculations to follow are derived from the frequency of the *Duffy* phenotypes of unrelated English

TABLE 1

The Duffy *blood groups of 110 English families with 274 children*

Group number	Father	Mother	Number of families	Children	
				Fy(*a*+)	*Fy*(*a*−)
1	*Fy*(*a*+)	*Fy*(*a*+)	8	1	—
			18	2	—
			5	3	—
			1	4	—
2	*Fy*(*a*+)	*Fy*(*a*+)	5	1	1
			3	1	2
			1	2	1
			1	3	1
			1	3	2
			1	6	2
3	*Fy*(*a*+)	*Fy*(*a*−)	1	1	—
			3	2	—
			3	3	—
			2	6	—
	Fy(*a*−)	*Fy*(*a*+)	3	1	—
			4	2	—
			1	4	—
			1	5	—
4	*Fy*(*a*+)	*Fy*(*a*−)	1	—	1
			4	—	2
			4	1	1
			2	1	2
			1	2	2
			1	3	2
	Fy(*a*−)	*Fy*(*a*+)	3	—	1
			3	—	2
			5	1	1
			1	—	3
			3	1	2
			4	2	1
			2	1	3
			1	2	3
5	*Fy*(*a*−)	*Fy*(*a*−)	6	—	2
			5	—	3
			1	—	4
			1	—	5

people (table 2). The item " present series " represents tests done in this Unit on unrelated, unselected persons since the previous publication.

Assuming that the phenotype *Fy*(*a*−) represents the genotype Fy^bFy^b then the gene frequencies may be derived in the following way :

the frequency of the gene $Fy^b = \sqrt{0{\cdot}3401} = 0{\cdot}5832$

and the frequency of the gene $Fy^a = 1 - 0{\cdot}5832 = 0{\cdot}4168$

TABLE 2

The frequency of the Duffy *phenotypes in England*

	Number tested	*Fy*(*a*+)	*Fy*(*a*−)
Cutbush and Mollison (1950)	205	133 *64·88%*	72 *35·12%*
Race, Holt and Thompson (1951)	255	167 *65·49%*	88 *34·51%*
Present series	325	218 *67·08%*	107 *32·92%*
Total	785	518 *65·99%*	267 *34·01%*

The genotype frequencies are therefore :—

$$Fy^aFy^a \quad 0{\cdot}4168^2 = 0{\cdot}1737$$
$$Fy^aFy^b \quad 0{\cdot}4168 \times 0{\cdot}5832 \times 2 = 0{\cdot}4862$$
$$Fy^bFy^b \quad 0{\cdot}5832^2 = 0{\cdot}3401$$

These frequencies have been used to analyse the family material in two ways.

METHOD 1

Table 3 shows the expected incidence of the various matings and the relative frequency of *Fy*(*a*+) and *Fy*(*a*−) children of these matings. These expectations are applied in table 4 to the 110 families and their 274 children. It will be seen that there is good agreement between the calculated distribution and that actually found.

Professor Fisher has pointed out certain objections to this method which has almost become the standard way of verifying unifactorial inheritance. The mating *Fy*(*a*+) × *Fy*(*a*+) is genotypically of three kinds, and the mating *Fy*(*a*+) × *Fy*(*a*−) of two kinds. Children from say the phenotype mating *Fy*(*a*+) × *Fy*(*a*−) are not independent samples from a homogeneous population, with an expectation of 0·6316 *Fy*(*a*+) to 0·3684 *Fy*(*a*−), and should not be treated as independent in calculating goodness of fit. That this method does in fact usually give a very good fit is probably due to the shortage of large families in the data.

METHOD 2 (*a*)

In 1939 Professor Fisher suggested that account should be taken separately of the totals yielded by the group of families containing recessive children, and of the individual sizes of families containing none. This method was applied to the A_1 A_2 B O groups by Taylor and Prior (1939) and by Race, Ikin, Taylor and Prior (1942). Owing to an unfortunate lapse of memory the method was not referred to in *Blood Groups in Man* (Race and Sanger, 1950).

Two separate comparisons with expectation are made. In (*a*), we compare the number of families observed to contain no recessive children, irrespective of size, with the number expected, taking account

of the estimated gene frequency and of the sizes of all families observed. In (*b*), we compare the numbers of recessive children observed in

TABLE 3

The expected distribution of the Duffy *groups in English parents and offspring*

Genotypes				
Matings		Children		
Type	Frequency	Fy^aFy^a	Fy^aFy^b	Fy^bFy^b
$Fy^aFy^a \times Fy^aFy^a$	*0·0302*	0·0302	...	...
$Fy^aFy^a \times Fy^aFy^b$	*0·1689*	0·08445	0·08445	...
$Fy^aFy^b \times Fy^aFy^b$	*0·2364*	0·0591	0·1182	0·0591
$Fy^aFy^a \times Fy^bFy^b$	*0·1181*	...	0·1181	...
$Fy^aFy^b \times Fy^bFy^b$	*0·3307*	...	0·16535	0·16535
$Fy^bFy^b \times Fy^bFy^b$	*0·1157*	...	...	0·1157
	1·0000			

Phenotypes			
Matings		Children	
Type	Frequency	*Fy*(a+)	*Fy*(a−)
Fy(a+) × *Fy*(a+)	*0·4355*	0·8643	0·1357
Fy(a+) × *Fy*(a−)	*0·4488*	0·6316	0·3684
Fy(a−) × *Fy*(a−)	*0·1157*	...	1·0000
	1·0000		

TABLE 4

The Duffy *groups of 110 English families with 274 children analysed by method 1*

Matings			Children				
Type	Number		Total number	*Fy*(a+)		*Fy*(a−)	
	Observed	Expected		Observed	Expected	Observed	Expected
Fy(a+) × *Fy*(a+)	44	*47·9*	102	85	*88·2*	17	*13·8*
Fy(a+) × *Fy*(a−)	53	*49·4*	136	79	*85·9*	57	*50·1*
Fy(a−) × *Fy*(a−)	13	*12·7*	36	0	*0·0*	36	*36·0*
	110	*110·0*	274				

those families which contain any, with the numbers expected on Mendelian theory, when allowance has been made for the fact that

at this stage, each of these families is known to contain at least one recessive child.

The formulæ given by Taylor and Prior (1939) for the matings $B \times O$ and $B \times B$ can be directly applied to matings $Fy(a+) \times Fy(a-)$ and $Fy(a+) \times Fy(a+)$. These formulæ are also applicable to family studies of the *P*, *Lutheran* and *Kidd* blood groups where

a represents the gene frequency of Fy^a (or *P*, Lu^a or Jk^a)
and b represents the gene frequency of Fy^b (or *p*, Lu^b or Jk^b)

The explanation given below is almost a direct transcription from Taylor and Prior's lucid account of the method, with the necessary change of symbols.

Mating Fy(a+)×Fy(a−)

The $Fy(a+)$ parent can be one of two genotypes, Fy^aFy^a, the frequency of which is a^2, or Fy^aFy^b with the frequency of $2ab$. The frequency of the phenotype $Fy(a+)$ in the population is therefore a^2+2ab; hence the probability of an $Fy(a+)$ person being Fy^aFy^a is $\frac{a^2}{a^2+2ab}$ or $\frac{a}{a+2b}$, and of being Fy^aFy^b is $1-\frac{a}{a+2b}$. From the mating $Fy^aFy^a \times Fy^bFy^b$ all children will be $Fy(a+)$, from the mating $Fy^aFy^b \times Fy^bFy^b$ some children may be $Fy(a-)$. The probability of a child of the mating $Fy^aFy^b \times Fy^bFy^b$ being of the phenotype $Fy(a+)$ is $\frac{1}{2}$, of two children being both $Fy(a+)$ is $(\frac{1}{2})^2$ and of n children all $Fy(a+)$ is $(\frac{1}{2})^n$. The occurrence of an $Fy(a-)$ child is at present the only means of knowing that the $Fy(a+)$ parent is heterozygous, hence in the mating $Fy(a+) \times Fy(a-)$ the families are divided into two groups, those with all children $Fy(a+)$, and those with some children $Fy(a-)$.

All children Fy(a+)

Families of 1 child.—Probability of this child being $Fy(a+)$ = probability of the $Fy(a+)$ parent being $Fy^aFy^a + \frac{1}{2} \times$ (probability of the $Fy(a+)$ parent being Fy^aFy^b) $= \frac{a}{a+2b} + \frac{1}{2}\left(1 - \frac{a}{a+2b}\right)$

Therefore the expected number of families with one child, this child being $Fy(a+)$

$$= \begin{bmatrix}\text{observed number of } Fy(a+) \times Fy(a-) \\ \text{families with one child.}\end{bmatrix} \times \left[\frac{a}{a+2b} + \frac{1}{2}\left(1 - \frac{a}{a+2b}\right)\right]$$

Since a = the frequency of the gene $Fy^a = 0{\cdot}4168$, and b = the frequency of the gene $Fy^b = 0{\cdot}5832$ and the total number of 1 child families of the mating type $Fy(a+) \times Fy(a-)$ is 8, then the expected number of such families with 1 child, this child being $Fy(a+)$ is $8 \times 0{\cdot}6316$ or $5{\cdot}0528$; 4 were observed.

Families of n *children.*—Probability of all *n* children being

$$Fy(a+) = \frac{a}{a+2b} + \left(\frac{1}{2}\right)^n \left(1 - \frac{a}{a+2b}\right)$$

As above, to get the expected number of such families this figure must be multiplied by the observed number of families of *n* children with parents $Fy(a+) \times Fy(a-)$.

TABLE 5

Analysis of families of the mating type Fy(a+)×Fy(a−)

Family size	Total families	Number of families having only *Fy*(*a*+) children	
		Expected	Observed
1	8	5·05	4
2	23	10·29	7
3	13	4·62	3
4	4	1·24	1
5	3	0·86	1
6	2	0·55	2
Total . .	53	22·61	18

Applying these formulæ to the matings $Fy(a+) \times Fy(a-)$ shown in table 1, the expectations for the number of families in which all children are $Fy(a+)$ are as shown in table 5.

Some children Fy(a−)

The expected number of families with $Fy(a-)$ children is obtained by subtracting the expected number of families whose children are all $Fy(a+)$ from the total number of observed families of the type $Fy(a+) \times Fy(a-)$. Applying this to the present investigation we find that the expected number of such families is 53—22·61 or 30·39, while 35 were observed.

Mating Fy(a+)×Fy(a+)

In this mating also the parents can be of two genotypes Fy^aFy^a or Fy^aFy^b and the families are again divided into two groups, those with all children $Fy(a+)$, and those with some children $Fy(a-)$.

Probability of one parent being $Fy^aFy^b = \frac{2b}{a+2b}$.

Probability of both parents being $Fy^aFy^b = \left(\frac{2b}{a+2b}\right)^2$.

Thus the probability of at least one parent being

$$Fy^aFy^a = 1 - \left(\frac{2b}{a+2b}\right)^2.$$

All children Fy(a+)

Families of 1 child.—Probability of this child being $Fy(a+)$ = the probability of at least one parent being $Fy^aFy^a + \frac{3}{4} \times$ (the probability of both parents being Fy^aFy^b) $= 1 - \left(\frac{2b}{a+2b}\right)^2 + \frac{3}{4}\left(\frac{2b}{a+2b}\right)^2$

Therefore the expected number of families with 1 child, this child being $Fy(a+)$

$$= \begin{bmatrix}\text{observed number of } Fy(a+) \times Fy(a+) \\ \text{families with 1 child.}\end{bmatrix} \times \left[1 - \left(\frac{2b}{a+2b}\right)^2 + \frac{3}{4}\left(\frac{2b}{a+2b}\right)^2\right]$$

Families of n *children.*—Probability of all children being

$$Fy(a+) = 1 - \left(\frac{2b}{a+2b}\right)^2 + \left(\frac{3}{4}\right)^n \left(\frac{2b}{a+2b}\right)^2$$

Applying these formulæ to the matings $Fy(a+) \times Fy(a+)$ shown in table 1 the expectations for the number of families in which all children are $Fy(a+)$ are as given in table 6.

TABLE 6

Analysis of families of the mating type Fy(a+)×Fy(a+)

Family size	Total families	Number of families having only $Fy(a+)$ children	
		Expected	Observed
1	8	*6·91*	8
2	23	*17·54*	18
3	9	*6·17*	5
4	2	*1·26*	1
5	1	*0·59*	0
8	1	*0·51*	0
Total . .	44	*32·98*	32

Some children Fy(a−)

The expected number of families with $Fy(a-)$ children will be the difference between the total number of observed families of the type $Fy(a+) \times Fy(a+)$ and the expected number of such families whose children are all $Fy(a+)$. In the present example the expected number of such families is 44—32·98 or 11·02, while 12 were observed.

A summary of the results of this method of family analysis is given in table 7.

METHOD 2 (*b*)

In the families $Fy(a+) \times Fy(a+)$ with a child $Fy(a-)$ it is possible to calculate the additional number of $Fy(a-)$ children expected.

Table 1 shows that there are 12 such matings with 22 *Fy*(*a*+) children and 5 additional *Fy*(*a*−) children (after the 12 diagnostic children have been excluded). The expected number of additional *Fy*(*a*−) children is simply $\frac{1}{4}(22+5)$ or 6·75.

TABLE 7

Analysis of certain of the families by method 2 (a)

Class of mating	Class of family	Expected number of families	Observed number of families	χ^2	d.f.
Fy(*a*+)×*Fy*(*a*+)	All children *Fy*(*a*+)	32·98	32	0·1167	1
	Some children *Fy*(*a*−)	11·02	12		
Fy(*a*+)×*Fy*(*a*−)	All children *Fy*(*a*+)	22·61	18	1·6373	1
	Some children *Fy*(*a*−)	30·39	35		
				1·7540	2
				probability = 0·40	

Similarly there are 35 families *Fy*(*a*+)×*Fy*(*a*−) with a child *Fy*(*a*−). In these families there are 31 *Fy*(*a*+) and 22 additional *Fy*(*a*−) children (again after the exclusion of the 35 diagnostic children). The expected number of additional *Fy*(*a*−) children is $\frac{1}{2}(31+22)$ or 26·50.

The analysis by Method 2 (*b*) is summarised in table 8.

TABLE 8

Analysis by method 2 (b) of children from families containing Fy(a−) *children*

Matings		Children			
		Fy(*a*+)	Additional *Fy*(*a*−)	χ^2	d.f.
Fy(*a*+)×*Fy*(*a*+)	Expected	20·25	6·75	0·6049	1
	Observed	22	5		
Fy(*a*+)×*Fy*(*a*−)	Expected	26·50	26·50	1·5283	1
	Observed	31	22		
				2·1332	2
				probability = 0·34	

The purpose of such analyses as those described in this paper is in the first place to disclose how a newly discovered blood group system is inherited. Conversely, once the manner of inheritance is established beyond doubt, good agreement between calculated and observed frequencies encourages confidence that the tests are being properly done. Furthermore, though the way of inheritance of a blood group system may be known with certainty, statistical analysis may reveal unexpected complications, such as for example, a differential survival rate.

SUMMARY

The results are given of testing a further 325 unrelated English persons with anti-Fy^a. These bring the published number of such persons tested to 785, of which 518 or 65·99 per cent. were positive. This corresponds to the gene frequencies $Fy^a = 0{\cdot}4168$ and $Fy^b = 0{\cdot}5832$.

The *Duffy* blood groups of a further 52 families are presented; these families together with 58 previously reported from this Unit are submitted to statistical analysis. A method of analysis for the $A_1\ A_2\ B\ O$ groups, suggested by Fisher in 1939, is applied to the *Duffy* system. The family results are in good agreement with those expected on the assumption that the antigen Fy^a is controlled by a gene which expresses itself in single as well as in double dose.

Acknowledgments.—The authors have to thank Professor Fisher for any originality which this paper may contain.

For gifts of the serum anti-Fy^a we are indebted to Dr P. L. Mollison and Miss Marie Cutbush of the Medical Research Council Blood Transfusion Research Unit, to Dr P. Vogel and Dr R. E. Rosenfield of New York and to Dr J. J. van Loghem, Jr. of Amsterdam. For most of the other anti-sera used in the investigation we are indebted to Dr A. E. Mourant of the Medical Research Council Blood Group Reference Laboratory.

We are particularly grateful to the members of the families for their blood, and for their encouraging interest and cheerful tolerance of our intrusion.

REFERENCES

CUTBUSH, MARIE, AND MOLLISON, P. L. 1950. The *Duffy* blood group system. *Heredity*, *4*, 383-389.

CUTBUSH, MARIE, MOLLISON, P. L., AND PARKIN, DOROTHY M. 1950. A new human blood group. *Nature*, *165*, 188.

HOLT, HELENE A., THOMPSON, JOAN S., SANGER, RUTH, AND RACE, R. R. 1952. Linkage relations of the blood group genes of man: an analysis of 487 families with two or more children. *Heredity*, in the Press.

RACE, R. R., HOLT, HELENE A., AND THOMPSON, JOAN S. 1951. The inheritance and distribution of the *Duffy* blood groups. *Heredity*, *5*, 103-110.

RACE, R. R., IKIN, ELIZABETH W., TAYLOR, G. L., AND PRIOR, AILEEN M. 1942. A second series of families examined in England for the $A_1\ A_2\ B\ O$ and *MN* blood group factors. *Ann. Eugen.*, Lond., *11*, 385-394.

RACE, R. R., AND SANGER, RUTH. 1950. *Blood Groups in Man.* Oxford: Blackwell Scientific Publications.

TAYLOR, G. L., AND PRIOR, AILEEN M. 1939. Blood groups in England. III. Discussion of the family material. *Ann. Eugen.*, Lond., *9*, 18-44.

THE ESTIMATION OF LINKAGE WITH INCOMPLETE PENETRANCE

ENRIQUE SÁNCHEZ-MONGE
Estación Experimental de Aula Dei, Zaragoza, Spain

Received 11.ix.51

PENETRANCE of a gene is measured as the proportion of the individuals carrying this gene which are phenotypically different from the individuals of the population carrying the allele.

The problem discussed in the following is that of the estimation of linkage between two genes, one of which has incomplete penetrance. Tests of significance of the estimates of linkage and penetrance are also given.

We will treat separately the case of backcross data and the case of double heterozygotes intercross or selfing data.

1. BACKCROSS DATA

The offspring of a backcross $A\alpha B\beta \times \alpha\alpha\beta\beta$, supposing linkage in coupling phase and p being the recombination fraction, falls in four phenotypic classes: AB, Ab, aB, ab, the expected frequencies being those given in table 1 under $u = 1$. The total of individuals is n.

Supposing now that a fraction u of the Aa genotype is classified phenotypically as aa, the expected frequencies will be those given in table 1 under $u \neq 1$.

TABLE 1

Phenotype	Expected frequencies $\times \frac{2}{n}$	
	$u = 1$	$u \neq 1$
AB	$1-p$	$(1-p)u$
Ab	p	pu
aB	p	$p+(1-p)(1-u)$
ab	$1-p$	$1-p+p(1-u)$

Writing $(1-p)\ u = x$ and $pu = y$, the relative frequencies of the phenotypes AB, Ab, aB, ab will be respectively x, y, $1-x$, $1-y$, and if a, b, c, d are the observed numbers $(a+b+c+d = n)$, the likelihood of obtaining the observed family is represented by (see Fisher, 1921)

$$\frac{n!}{a!\,b!\,c!\,d!}\, x^a y^b (1-x)^c (1-y)^d \quad . \quad . \quad . \quad (1)$$

The method of maximum likelihood depends on the maximization of this expression with respect to x and y. Its differentiation being difficult we use its logarithm that will be made maximum by the same values of x and y.

Differentiating (1) logarithmically we obtain :

$$\frac{\delta L}{\delta x} = \frac{a}{x} - \frac{c}{1-x} \quad . \quad . \quad . \quad . \quad (2)$$

$$\frac{\delta L}{\delta y} = \frac{b}{y} - \frac{d}{1-y} \quad . \quad . \quad . \quad . \quad (3)$$

Equating (2) and (3) to zero, the values of x and y will be :

$$x = \frac{a}{a+c} \quad , \quad y = \frac{b}{b+d} \quad . \quad . \quad . \quad (4)$$

Therefore :

$$u = x+y = \frac{a}{a+c} + \frac{b}{b+d} \quad . \quad . \quad . \quad . \quad (5)$$

$$p = \frac{y}{u} = \frac{b(a+c)}{a(b+d)+b(a+c)} \quad . \quad . \quad . \quad (6)$$

If we wish to test the significance of the departure of u from unity, admitting the linkage, we have that the maximum likelihood equation for $u = 1$ gives $p = \frac{1}{n}(b+c)$ and the expectations will be :

$$\tfrac{1}{2}(a+b) \quad , \quad \tfrac{1}{2}(b+c) \quad , \quad \tfrac{1}{2}(b+c) \quad , \quad \tfrac{1}{2}(c+d)$$

which compared with a, b, c, d give :

$$\chi^2 = \frac{(a-d)^2}{a+d} + \frac{(b-c)^2}{b+d} \quad . \quad . \quad . \quad (7)$$

Of the three available degrees of freedom one has been used in the estimation of p, leaving two for testing the significance of $u \neq 1$.

Conversely, to detect the existence of linkage, admitting incomplete penetrance, the value of u for $p = \frac{1}{2}$ is $u = \frac{2}{n}(a+b)$, and the expectations will be :

$$\tfrac{1}{2}(a+b) \quad , \quad \tfrac{1}{2}(a+b) \quad , \quad \tfrac{1}{2}(c+d) \quad , \quad \tfrac{1}{2}(c+d)$$

which compared with a, b, c, d give :

$$\chi^2 = \frac{(a-b)^2}{a+b} + \frac{(c-d)^2}{c+d} \quad . \quad . \quad . \quad . \quad (8)$$

with two degrees of freedom, as one is lost in the estimation of u.

For the calculations of the variance of u and p, we can use the formula (see Fisher, 1946) :

$$V_u = \Sigma\left[m\left(\frac{\delta u}{\delta a}\right)^2\right] - n\left(\frac{\delta u}{\delta n}\right)^2 \quad . \quad . \quad . \quad (9)$$

where m are the expectations corresponding to the observed values a, b, c, d.

Thus we derive :

$$V_u = \frac{n}{2}\left[\frac{ac}{(a+c)^4} + \frac{bd}{(b+d)^4}\right] \quad . \quad . \quad . \quad (10)$$

An easy way to calculate V_p is to write

$$z = L\frac{1-p}{p} = L\frac{a(b+d)}{b(a+c)}$$

and then to use (9) to evaluate V_z. We then have

$$V_z = \frac{n}{2}\left[\frac{c}{a(a+c)^2} + \frac{d}{b(b+d)^2}\right]$$

and V_p being (see Fisher, 1947)

$$V_p = V_z \frac{1}{\left(\frac{dz}{dp}\right)^2} \quad . \quad . \quad . \quad . \quad (11)$$

we have :

$$V_p = \frac{np^2(1-p)^2}{2}\left[\frac{c}{a(a+c)^2} + \frac{d}{b(b+d)^2}\right] \quad . \quad . \quad (12)$$

2. INTERCROSS DATA

In a cross of the type A*a*B*b* × A*a*B*b* with complete penetrance of both genes the frequencies of the four phenotypes in the offspring are those given in table 2 under $v = 1$; p and p' are the recombination fractions for male and female gametogenesis, giving $P = pp'$ for matings in repulsion and $P = (1-p)(1-p')$ for matings in coupling. But if penetrance of gene A is incomplete, so that a fraction v of the AA and A*a* genotypes are classified phenotypically as *aa*, the frequencies will be those given in table 2 under $v \neq 1$.

TABLE 2

Phenotypes	Frequencies × $\frac{4}{n}$	
	$v = 1$	$v \neq 1$
AB	2+P	(2+P)v
A*b*	1−P	(1−P)v
*a*B	1−P	1−P+(2+P)(1−v)
ab	P	P+(1−P)(1−v)

Writing $(2+P)v = x$, and $(1-P)v = y$, the relative frequencies will be $x, y, 3-x, 1-y$, and if a, b, c, d, are the corresponding observed numbers $(a+b+c+d = n)$, the logarithm of the likelihood expression will be :

$$L = K+aLx+bLy+cL(3-x)+dL(1-y)$$

Differentiating and equating to zero we obtain the maximum likelihood equations :

$$\frac{\delta L}{\delta x} = \frac{a}{x} - \frac{c}{3-x} = 0 \quad . \quad . \quad . \quad . \quad (13)$$

$$\frac{\delta L}{\delta y} = \frac{b}{y} - \frac{d}{1-y} = 0 \quad . \quad . \quad . \quad . \quad (14)$$

Then :

$$x = \frac{3a}{a+c} \quad ; \quad y = \frac{b}{b+d}$$

Therefore :

$$v = \frac{1}{3}(x+y) = \frac{a}{a+c} + \frac{b}{3(b+d)} \quad . \quad . \quad . \quad (15)$$

$$P = 1 - \frac{y}{v} = \frac{ab+3ad-2bc}{4ab+3ad+bc} \quad . \quad . \quad . \quad . \quad (16)$$

The significance of the discrepance between v and unity, admitting linkage, can be tested by a χ^2 obtained by comparing the actual numbers a, b, c, d with the frequencies obtained supposing $v = 1$. This χ^2 has two degrees of freedom as one is used for the calculation of P.

Conversely, in order to detect the existence of linkage, admitting $v \neq 1$, we make $P = \frac{1}{4}$, $\left(p = p' = \frac{1}{2}\right)$, and the value of v will be :

$$v = \frac{4(a+b)}{3n}$$

The expected numbers are then :

$$\frac{3}{4}(a+b) \quad , \quad \frac{1}{4}(a+b) \quad , \quad \frac{3}{4}(c+d) \quad , \quad \frac{1}{4}(c+d)$$

which compared with the observed numbers a, b, c, d, give :

$$\chi^2 = \frac{(3b-a)^2}{3(a+b)} + \frac{(3d-c)^2}{3(c+d)} \quad . \quad . \quad . \quad (17)$$

For the calculation of V_v we can use (9), and then we have :

$$V_v = \frac{n}{4}\left[\frac{3ac}{(a+c)^4} + \frac{bd}{9(b+d)^4}\right] \quad . \quad . \quad . \quad (18)$$

Now if we write

$$z = L\frac{1-P}{2+P} = L\,\frac{b(a+c)}{3a(b+d)}$$

we have :

$$V_z = \frac{n}{4}\left[\frac{3c}{a(a+c)^2}+\frac{d}{b(b+d)^2}\right] \quad . \quad . \quad . \quad (19)$$

and then

$$V_P = V_z \frac{1}{\left(\frac{dz}{dP}\right)^2} = \frac{n(1-P)^2(2+P)^2}{36}\left[\frac{3c}{a(a+c)^2}+\frac{d}{b(b+d)^2}\right] \quad . (20)$$

If the recombination fraction is the same in both sexes, $p = p'$, we obtain p from $P = (1-p)^2$, and V_p from :

$$V_p = V_P \frac{1}{\left(\frac{dP}{dp}\right)^2} \quad ; \quad \frac{dP}{dp} = 2(p-1)$$

thus :

$$V_p = \frac{V_P}{4(p-1)^2} = \frac{n(2p-p^2)^2(3-2p+p^2)^2}{144(p-1)^2}\left[\frac{3c}{a(a+c)^2}+\frac{d}{b(b+d)^2}\right] . (21)$$

3. REFERENCES

FISHER, R. A. 1921. On the mathematical foundations of theoretical statistics. *Phil. Trans. Roy. Soc. A., 122*, 309-368.

FISHER, R. A. 1946. *Statistical Methods for Research Workers.* 10th ed. London.

FISHER, R. A. 1947. *The Design of Experiments.* 4th ed. London.

COMMENT AND REVIEWS

THE EFFECT OF FRACTIONAL X-RAY DOSAGE ON THE FREQUENCY OF CHROMOSOME ABERRATIONS

KARL SAX

Bussey Institute, Harvard University

and

HENRY LUIPPOLD

University of Massachusetts

Received 23.x.51

The effect of fractional dosage and radiation intensity on the frequency of induced chromosome aberrations has provided information of value in interpreting the nature of chromosome breaks and reunions. It has been found that fractional dosage of prophase chromosomes in *Tradescantia* microspores decreases the frequency of 2-hit aberrations (translocations, dicentrics and rings), but has no effect on the frequency of 1-hit aberrations (chromatid and iso-chromatid deletions) (Sax, 1941). Fractional dosage or low intensities also decrease the frequency of 2-hit chromosome aberrations induced in the resting chromosomes of *Tradescantia* microspores (Sax, 1940, 1941). These and other observations have been used by Lea (1946) and Catcheside (1948) to explain the nature of X-ray induced chromosome breaks and reunions. They show that the breaks necessary to permit the formation of a 2-hit chromatid or chromosome aberration must be limited in both time and space, and that many of the breaks undergo restitution.

Recently Lane (1951) has questioned the validity of the author's technique and results with fractional dosage. The technique of the earlier work has been questioned on the grounds that the temperature was not held constant between the time of irradiation and fixation of the microspores, and that cells fixed 24 hours after irradiation are excessively variable due to " relatively large effects of environmental variation." It is true that temperature does affect the frequency of X-ray induced chromosome aberrations (Sax and Enzmann, 1939), but the effect is limited to the period during and shortly after irradiation, and the normal laboratory temperature range would have relatively little effect. There is, of course, some variation in aberration frequency which might be attributed to " environmental variation," but we have found no evidence that this variation is any greater in prophase than in the resting stage. It is more difficult to score chromatid aberrations than chromosome aberrations, but in either case adequate numbers of observations have given consistent results.

Lane's experiments covered three growing seasons (1947, 1948 and 1949). Presumably he did not have a clonal line of *Tradescantia* which can be flowered throughout the year in a greenhouse. The irradiation was done at 18° C. and the inflorescences were held at 24° C. between irradiation and fixation. The X-ray dosage was 360 *r* given at the rate

of 25 *r* per minute in the more extensive experiments. The first year's work was too limited to be of any value. In 1948 it was found that two exposures of 180 *r* each separated by a rest period of 4 hours reduced the 2-hit aberration frequency, but only 480 chromosomes were analysed in each of the six experiments. In 1949 fractional dosage with rest periods of 1, 2, 4, 6 and 8 hours respectively showed a reduction in aberration frequency as the rest period increased up to and including 4 hours, but aberration frequency began to increase with the 6-hour rest period, and with an 8-hour interval between the two exposures the aberration frequency (9·0 per cent.) was nearly as high as that resulting from a single 360 exposure (9·9 per cent.). Only 960 chromosomes were analysed in each series of experiments with the exception of the single dose of 180 *r* where 1920 chromosomes were recorded.

These results led Lane to conclude that the decrease in aberration frequency following fractional dosage is due to physiological changes in the cell which makes the chromosomes less sensitive to the second exposure when the rest period is less than 8 hours. With an 8-hour rest period the cell has recovered from the first exposure and two doses of 180 *r* each then produce nearly as many aberrations as does a single dose of 360 *r*. Presumably it takes some time for the alleged physiological effect to become effective, since increasing rest periods up to 4 hours show an increasing decline in the frequency of aberrations. It is also claimed that two doses of 180 *r* each separated by a 4-hour rest period produces fewer aberrations than do two independent 180 *r* exposures.

Shortly after Lane's paper became available, a series of experiments were started for an analysis of the effects of fractional dosage, using the same dosage and rest periods used by Lane. The inflorescences of *Tradescantia paludosa* (Clone 3) were rayed at 180 *r* per minute, since at 25*r*/min. the aberration frequency is reduced by the intensity effect. After irradiation the inflorescences were placed in a greenhouse. Microspores were fixed on the fifth day after irradiation. An interval of only 2 or 3 days between irradiation and fixation often results in some chromatid aberrations as Lane has found. The control of temperature was not considered necessary because each series of experiments included an exposure of 360 *r* without fractionation which served as a control.

In the analysis of chromosome aberrations only centric rings and dicentrics were included. Dot deletions were frequent, but were not included because as Rick (1940) has shown they are a mixture of 1-hit and 2-hit aberrations. The simple terminal deletions were not included because they occur with such a low frequency that significant results could be attained only by analysing enormous numbers of chromosomes. A series of three experiments were completed in August and September, including in each one a single 360 *r* exposure, a fractionate dose of 360 *r* with rest periods of 4, 8 or 12 hours, and a single exposure of 180 *r*. The results of the single exposures were similar in all cases and the results of the three series have been combined. The data are shown in table 1.

The single dose of 360 *r* produced a total of 2055 dicentric and ring chromosomes or 12·2 per cent. compared with Lane's results of 9·9 per cent. The difference can be attributed to the higher intensity of radiation (180 *r*/min.) than that used by Lane (25 *r*/min.). A 360 *r* dose at 18 *r*/min.

gave essentially the same results as were obtained by Lane. Two exposures of 180 *r* each separated by rest periods of 4, 8 and 12 hours respectively produced essentially the same aberration frequency. The fractionated dose

TABLE 1

Effect of fractional dosage on frequency of 2-hit Chromosome aberrations. 180 r/min.

Dose	Rest period	N-chromosomes	Dic and Ring	Per cent.
360	0	16,848	2055	12·2
180+180	4 hrs.	17,820	1534	8·6
180+180	8 hrs.	9,990	847	8·5
180+180	12 hrs.	6,000	487	8·1
180	0	16,900	726	4·3

with a 12-hour rest period produced a somewhat lower frequency of aberrations than did the fractionated doses with 4- or 8-hour rest periods, but only 6000 chromosomes were analysed in this case. There is no evidence of an increase of aberration frequency as the rest period is increased to 8 or 12 hours. A single exposure of 180 *r* produced 4·3 per cent. of dicentrics and rings, or essentially half of the values from the 360 *r* fractionated exposures.

These experimental results, based upon repeated experiments and adequate numbers of chromosomes, confirm earlier conclusions. Fractional dosage does decrease the frequency of 2-hit chromosomal aberrations even with rest periods of as much as 12 hours. There is no evidence of a physiological effect of radiation which reduces breakage of chromosomes. Nor is there any evidence that long rest periods restore radiation sensitivity. The earlier interpretation appears valid. Fractional dosage reduces the aberration frequency because of restitution of single breaks during the rest period. If the rest period is very short the simple breaks surviving the first exposure persist until breaks are induced at the second exposure when these breaks become involved in the production of dicentric and ring chromosomes and other 2-hit aberrations. With longer rest periods the breaks which have not undergone restitution or illegitimate reunion during the first exposure do so during the rest period so that no breaks survive to take part in the aberrations induced during the second exposure. Under such circumstances two fractionated doses should not be any more effective than two separate doses. With four or more hours of rest between the two 180 *r* exposures the frequency of 2-hit aberrations does not exceed twice the aberration frequency induced by a single exposure of 180 *r*. Nor is there any significant decline of aberration frequency below this base line. The base line ($2 \times 4{\cdot}3 = 8{\cdot}6$) does not deviate significantly from the fractional dosage values with 4-, 8- or 12-hour rest periods. The value of 8·1 per cent. with the 12-hour rest period is somewhat below the base line, but this experiment involved only 6000 chromosomes. There was also considerable variation between 100 cell samples with the 180 *r* series, and even with an analysis of 16,900 chromosomes the aberration frequency of 4·3 per cent. might well have varied a few tenths of a per cent. in either direction.

These results are in accord with earlier experiments (Sax, 1941) with fractional dosage involving chromatid breaks where it was possible to

compare the effects on both 1-hit and 2-hit aberrations. It was found that fractional dosage reduced the frequency of 2-hit aberrations, but produced as many simple deletions as did a single dose. Similar differential effects on the frequency of 1-hit and 2-hit aberrations were also found by reducing radiation intensity. High intensities of X-rays produce a much higher frequency of 2-hit aberrations than do low intensities, but the production of 1-hit aberrations is independent of intensity. These results can best be explained on the limitations of breaks in time and space. If two adjacent breaks occur within certain limits of time or space it is possible to obtain a 2-hit aberration. Lea (1946) has calculated the space factor as about 1 micron and the time factor as quite variable but with an average value of 3 or 4 minutes.

Lane dismisses the absence of any decrease in frequencies of 1-hit aberrations following fractional doses or low intensities as due to excessive variability of the cells when rayed at prophase and given only a 24-hour interval between irradiation and fixation. He has, however, made no tests to confirm his assumption. Nor has he presented any valid explanation of the differences in relative frequencies of 2-hit aberrations following irradiation with X-rays and neutrons. Although the variation in sensitivity of the chromosomes to X-rays during the cell cycle has little bearing on the effects of fractional dosage, Lane attempts to explain the decreased sensitivity of the chromosomes following the first exposure as due to an increased nucleic acid charge induced by radiation. He accepts the assumption of Darlington and La Cour (1945) that increased accumulation of nucleic acid decreases sensitivity to X-rays, although there is ample evidence to the contrary (Sparrow, 1949; Bishop, 1950). Lane's results can best be attributed to his inadequate data and inadequate analysis.

SUMMARY

Repeated experiments have not confirmed Lane's (1951) conclusions that the reduction of chromosome aberration frequency following fractional dosage can be attributed to a " depressing effect on sensitivity to breakage." Fractional dosage of *Tradescantia* microspores results in essentially the same reduction of chromosome aberrations with 4-, 8- or 12-hour rest periods. With these rest periods between two exposures of 180 *r* each the aberration frequency is much less than that obtained with a single dose of 360 *r*, and is approximately twice that obtained from a single exposure of 180 *r*. The effect of fractional dosage can be attributed to the failure of breaks induced at the first exposure to survive the longer rest periods, so that broken ends induced at the first exposure cannot unite with broken ends induced at the second exposure to produce the 2-hit chromosome aberrations.

This work was supported by a grant from the Office of Naval Research, Contract No. N8-ONR-73100.

REFERENCES

BISHOP, C. J. 1950. Differential X-ray sensitivity of *Tradescantia* chromosomes during the mitotic cycle. *Genetics*, *35*, 175-187.

CATCHESIDE, D. G. 1948. Genetic effects of radiation. *Advances in Genetics*, *2*, 271-358.

DARLINGTON, C. D., AND LA COUR, L. F. 1945. Chromosome breakage and the nucleic acid cycle. *J. Genet.*, *46*, 180-267.

LANE, G. R. 1951. X-ray fractionation and chromosome breakage. *Heredity*, *5*, 1-35.

LEA, D. E. 1946. *Actions of Radiations on Living Cells*. Cambridge.

RICK, C. M. 1940. On the nature of X-ray induced deletions in *Tradescantia* chromosomes. *Genetics*, *25*, 466-482.

SAX, K. 1940. An analysis of X-ray induced chromosomal aberrations in *Tradescantia*. *Genetics*, *25*, 41-68.

SAX, K. 1941. Types and frequencies of chromosomal aberrations induced by X-rays. *Cold Spr. Harbor Symposium*, *9*, 93-103.

SAX, K., AND ENZMANN, E. V. 1939. The effect of temperature on the frequency of X-ray induced chromosome aberrations. *Proc. Nat. Acad. Sci. Wash.*, *25*, 397-405.

SPARROW, A. H. 1949. X-ray sensitivity changes in meiotic chromosomes and the nucleic acid cycle. *Proc. Nat. Acad. Sci. Wash.*, *30*, 147-155.

REMARKS ON SAX AND LUIPPOLD

Sax's new data confirm the general conclusion drawn from previous experiments, that fractionation of X-ray dose reduces the yield of chromosome reunions. But, for the following reasons, I cannot accept the conclusions which Sax draws from the discrepancy between his results and my own. Nor can I take the view that the new experiments throw further light on the cause of the reduction. In the first place, in the experiments which Sax compares with my own, the temperature at which the material develops is not controlled nor is it even recorded so far as we are told. In the second place, Sax employs different material, a different intensity and a different time period between irradiation and fixation.

Since temperature affects the rate of physiological processes, time alone without controlled temperatures cannot serve as a measure in the comparison of experiments in which physiological processes are involved. The usefulness of comparing time-scaled experiments in which the temperature is uncontrolled, particularly if the other conditions and the materials differ in the different experiments, is therefore open to question.

A comparison between some earlier experiments carried out by Sax (Sax, K., 1939, *P.N.A.S.*, *25*, 225-233) and the present experiments serves to illustrate this point. In these earlier experiments he observes that the rate of decrease in yields of aberrations with increasing intervals between fractions varies with the intensity. It is much greater at lower intensities of 25 *r*/min. or 50 *r*/min. than it is at 160 *r*/min. As Sax puts it " at high intensity, 160 *r*/m, there is little or no effect of the single rest period." These results are clearly pertinent to any comparison made between Sax's present experiments (intensity 180 *r*/min.) and mine (25 *r*/min.).

In discussing the temporary physiological effects of radiation (*e.g.* the effect on the rate of development and the effects on nucleic acid metabolism), I remarked that the timing and magnitude of these effects depend upon the dose and possibly the intensity, as well as upon the material used and the external conditions. If, as I have suggested, the reduction in breakage frequency with fractionation of dose is, in the main, the result of a physiological effect, the timing and magnitude of this effect would be expected to vary with all these factors. It is therefore not surprising that Sax's results differ from mine, but without a knowledge of the experimental conditions we are not in a position to attempt to interpret his results.

Further experiments will doubtless show to what extent my explanation of my own results is correct, but they can do so only if they fulfil the essential conditions referred to in my paper. Insufficiently controlled experiments can only confuse the issue. To carry out experiments which fulfil these conditions is not easy, but it is in my opinion the only way to shed further light on the matter.

G. Roy Lane.

REVIEWS

HUMAN FERTILITY : THE MODERN DILEMMA. By Robert C. Cook. (Introduction by Julian Huxley.) London : Gollancz. 1951. Pp. 348. 21s.

The numbers of mankind are greater and are increasing faster than ever before. This increase is, as hitherto, differential with respect to different races and classes. But the races and classes that are favoured are not, as hitherto, those technically *best* fitted to maintain themselves but those *least* fitted to do so. The limits of food production set by the area of cultivable land, and the capacity to cultivate it efficiently, have therefore been reached and passed for certain peoples. Thus the problems brought to light by Malthus and Galton in the eighteenth and nineteenth centuries have, after some delay, come into focus together in the twentieth. How they have done so, and the catastrophe with which they now threaten us, it is Robert Cook's purpose to explain. At the same time he attempts to show how the genetic and evolutionary principles of Mendel and Darwin were discovered and how they apply to the situation.

The presentation is popular, the style is expansive, and the phrasing not always rigorous. It does not mean anything, or at least it ought not to mean anything, to say that " four-fifths of human mental ability is traceable to genetic factors " (p. 212). And sometimes the statistics run loose—5 million immigrants into the U.S.A. is the maximum for 5 years, not for one year (p. 87). One wonders whether the death-rate in Italy is really 10·2 (p. 92) or lower than it has ever reached in England. And when it is said (p. 262) that in Japan : " In 1948 births exceeded deaths by nearly two million " should it not have been : 1,400,000 ? That, after all, is enough. It means 4000 a day, or a large and fully-loaded liner leaving every day for a foreign port, to keep the population stable. Or something greater than the maximum immigration rate into the U.S.A. in 1908.

These are details. The principles, the arguments and the conclusions are clear and, I believe, inescapable. Indeed they are devastating. Whether it is in the history of Puerto Rico, in the picture of the visiting ladies advising General MacArthur on the subject of birth control in Japan, or in the account of Lysenko expounding the true doctrine of heredity, we see the size of the task that confronts those who would apply their knowledge of biology to save humanity from a not very remote disaster.

C. D. D.

SCIENTIFIC THOUGHT IN THE TWENTIETH CENTURY. Edited by A. S. Heath. London : Watts. 1951. Pp. 387. 42s.

The articles in this book on Statistics by R. A. Fisher, Zoology by P. B. Medawar and Genetics by E. B. Ford will be of value to readers of *Heredity*. The attempts to describe Social Medicine by Alice Stewart, Sociology by D. G. Macrae, and especially Social Anthropology by Meyer Fortes,

without using the information or assumptions of genetics, also deserve our attention. Partly by accident and partly by intention they reveal the problems of what we may call the floating sciences. And they reveal them in the same book as those of the sciences to which (as Goldschmidt has so clearly explained in "Genetics in the 20th Century") they will have to be attached. C. D. D.

BOOKS RECEIVED

NOTES FOR INTRODUCTORY COURSES IN GENETICS. By C. Auerbach, Ph.D., D.Sc. London and Edinburgh : Oliver and Boyd. 1951. Pp. 41. 2s.

EUGENICS IN RETROSPECT AND PROSPECT. By C. P. Blacker, M.A., M.D. Occasional Papers on Eugenics. No. 1. 2nd ed. London : Cassell and Co. Ltd. 1950. Pp. 38. 2s. 6d.

PROBLEM FAMILIES IN BRISTOL. By R. C. Wofinden, M.D., D.P.H. Occasional Papers on Eugenics. No. 6. London : Cassell and Co. Ltd. 1950. Pp. 59. 2s. 6d.

THE EXPECTATION OF MENTAL INFIRMITY IN A SAMPLE OF THE DANISH POPULATION. By K. H. Fremming, M.D. Occasional Papers on Eugenics. No. 7. 1951. Pp. 53. 3s. 6d.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND SEVENTH MEETING of the Society, held on 17th NOVEMBER 1951, at the GALTON LABORATORY, University College, Gower Street, London, W.C. 1

A COMPENSATED SKELETAL ANOMALY IN THE MOUSE

HANS GRÜNEBERG

University College, London

Mice heterozygous for Danforth's short-tail (*Sd*/+) lack the odontoid process of the axis (dens epistrophei) and hence the pivot round which the head is normally turned from side to side. However, these animals have an atypical articulation between atlas and axis by means of which the turning movement of the head can be carried out. There is thus the extraordinary situation that a single gene substitution removes one type of joint and replaces it by another fully functional arrangement. It is assumed that the removal of the dens epistrophei is primary, and that the formation of an alternative kind of articulation is a mechanical consequence of the altered anatomical situation.

A NEW LINKAGE IN THE HOUSE MOUSE INVOLVING THE SEX CHROMOSOME

DONALD MICHIE

Department of Zoology, University of Oxford

CHROMOSOME ANALYSIS OF SELECTED STRAINS OF *DROSOPHILA*

FORBES W. ROBERTSON

Institute of Animal Genetics, Edinburgh

A number of inbred lines of *Drosophila melanogaster* have been established from strains selected for large and small body size. With the aid of marked inversions, genotypes can be created which include all possible combinations of the major chromosomes from pairs of contrasted strains. Such methods may be expected to throw light on the nature of the changes produced by selection, dominance relations and the extent to which the genetic background influences the effect of particular chromosome substitutions.

The analysis of two unrelated strains selected for small size has revealed substantial interaction between genes on different chromosomes.

SELECTION OF THE GENETIC BASIS OF AN ACQUIRED CHARACTER

C. H. WADDINGTON

Institute of Animal Genetics, Edinburgh

Developing flies of a wild type strain originally collected in Edinburgh were given a temperature shock by being placed at 40° C. for four hours at about 21-23 hours after pupation. A crossveinless phenocopy was produced with a frequency

of about 40 per cent. One selected line was started from the crossveinless flies, the phenocopies being bred from in each generation; in a second selected stock, breeding was from those which did not show the phenocopy. After 15 generations, the frequency of phenocopies had become over 90 per cent. and under 10 per cent. respectively. In the twelfth generation of the upwards selected stock, crossveinless flies appeared even among individuals to which the temperature shock had not been given. When these were bred from, the condition was certainly inherited, probably by a gene of incomplete penetrance whose behaviour is not yet fully worked out. Thus the crossveinless condition, initially produced as a response to an environmental stimulus, has during the course of selection picked up a genetic basis which enables it to appear in the absence of the stimulus.

PERITHECIUM ANALYSIS IN *ASPERGILLUS NIDULANS*

L. M. HEMMONS, G. PONTECORVO and A. W. S. BUFTON

Department of Genetics, University of Glasgow

The technique used since 1946 for the genetics of the self-fertile *A. nidulans* is based on the classification of segregants for a pair of alleles only among classes of recombinants for other marker genes. In a cross each ascus may originate from self karyogamy (A×A) or (B×B) or crossed karyogamy (A×B). There are some 10 to 100 thousand asci per perithecium. By dissecting out a number of asci from individual perithecia it was found that up to 85 per cent. of the perithecia carry asci all of one kind, either all (A×A) or all (B×B) or all (A×B), the remainder (" twins ") carrying asci of any two kinds. A new technique of genetic analysis —" perithecium analysis "—makes use of this fact. A sample of ascospores are plated on non-selective media from each of a number of perithecia using as markers genes controlling colour of conidia. One or more of the perithecia giving correct allele ratios for the markers are used for the complete classification as to other segregating genes. The application of this technique in a simplified form to 22 crosses, involving 22 *different* strains, has led to the detection of " relative heterothallism," *i.e.* the formation of crossed asci in excess of 50 per cent., the maximum theoretical in a cross between two completely homothallic strains. In 10 out of 22 crosses the proportion of crossed asci approached 100 per cent. Since the majority of the strains used (derived by mutation and recombination from three original self-fertile ones) are self-fertile, " relative heterothallism " implies preferential cross karyogamy. In the present case it has arisen in the laboratory, but it may occur in nature among other homothallic species; its identification is possible only by the use of markers.

THE ORIGIN OF NEW VARIETIES OF THE GARDEN CHRYSANTHEMUM

G. J. DOWRICK

John Innes Horticultural Institution, Bayfordbury

New varieties of *Chrysanthemum* arise either from seed or as bud sports. Chromosome numbers of the English varieties vary between 47-64 ($n = 9$) with two peaks at 54 and 61. These can be related to flower size. A similar variation was found by Shimotomai in Japanese varieties. Within certain varieties chromosome variation is found. The extent of this variation differs in different varieties and according to the environment. Various types of chromosome loss and gain are found which are responsible for this variation. In some families of vegetative sports varying in chromosome number, there is a difference in (i) colour, (ii) leaf shape and (iii) resistance to disease between the varieties. In their meiotic behaviour the hexaploid varieties normally have 27 bivalents and the unbalanced forms a minimum irregularity.

MALE STERILITY AS AN OUTBREEDING MECHANISM IN *ORIGANUM VULGARE*

D. LEWIS and L. K. CROWE

John Innes Horticultural Institution, Bayfordbury

An important feature of outbreeding mechanisms, such as separation of the sexes or incompatibility, is that the different sexes or mating groups contribute approximately equal amounts of chromatin to the progeny. Male sterility in plants is an exception in that the two sexes, female and hermaphrodite, contribute amounts of chromatin in a ratio of 1 : 3. If the male sterility is controlled by nuclear genes, then for this to evolve into an outbreeding mechanism the disparity of nuclear contribution must be offset by the female being substantially fitter than the hermaphrodite. Alternatively, if it is controlled by maternally inherited cytoplasm then this disparity of contribution of the sex determinants is avoided. Published work has shown that this type of control is not uncommon.

Origanum vulgare has male sterility as an integral part of its breeding system. Contrary to a published report that the control is cytoplasmic, data have been obtained which agree with a control by two major genes. A dominant gene causes anther abortion and another dominant gene is epistatic to the first and suppresses its action. The double recessive is lethal. A balance between the two sexes is obtained partly by this lethality but mainly by a greater fitness of the female. This is the first case of male sterility in wild populations which is controlled by major genes.

NATURAL CROSS-BREEDING IN WINTER BEANS

J. L. FYFE

School of Agriculture, Cambridge

Plants selected for high yield from a field of winter beans (*Vicia Faba* L.) were scored for hilum colour and in the following year their individual progenies were also scored. Assuming simple Mendelian inheritance of hilum colour, the proportions of self- and cross-pollination in the original crop were calculated. There was about 70 per cent. self-pollination but the distribution of genotypes among the selected plants showed no evidence of inbreeding, indicating that the highest yielding plants are cross-bred. Evidence was also obtained that adverse external influences bear more hardly on inbreds than on cross-breds.

It is suggested that the breeding system of a population of winter beans gives it a resilience by which, in responding to direct selection, it can to a considerable extent avoid far-reaching changes.

CONTRIBUTORS TO VOLUMES 1, 2, 3 AND 4

AUSTRALIA
- Burnett, F. M.
- Fenner, F.
- Montgomery, C.
- Smith-White, S.
- Walsh, R. J.

BELGIUM
- Heuts, M. J.

DENMARK
- Ditlevsen, E.
- Kemp, Tage
- Winge, Ø.

FINLAND
- Pesola, V. A.

FRANCE
- Ephrussi, B.
- L'Héritier, Ph.

GERMANY
- Pätau, K.
- Staudt, G.

INDIA
- Bhat, N. R.
- Majumdar, D. N.

ITALY
- Cavalli, L. L.
- Magni, G.
- Buzzati-Traverso, A.

JAPAN
- Kihara, H.

NEW ZEALAND
- Boyce, S. W.
- Copp, L. G. H.
- Frankel, O. H.
- Fraser, A. S.

NORWAY
- Berge, S.

PERU
- Harland, S. C.

SOUTH AFRICA
- Moffett, A. A.

SWEDEN
- Bonnier, G.
- Hansson, A.
- Müntzing, A.

TASMANIA
- Dobie, D. L.
- Ford, J. M.
- McAulay, A. L.

U.S.A.
- Dobzhansky, Th.
- Epling, C.
- Haskins, C. P.
- Haskins, E. F.
- Lederberg, J.
- Snell, G. D.

WEST INDIES
- Dodds, K. S.
- Simmonds, N. W.

UNITED KINGDOM
- Bateman, A. J.
- Billingham, R. E.
- Crane, M. B.
- Finney, D. J.
- Fisher, R. A.
- Ford, E. B.
- Koller, P. C.
- La Cour, L. F.
- Lewis, D.
- Mather, K.
- Medawar, P. B.
- Owen, A. R. G.
- Race, R. R.
- Yates, F.
- *and others*

Copies of Vols. 1, 2 and 3 are still available in 3 parts, royal 8vo. edges uncut price £3 or $10.50 net per volume

Vol. 1. Pp. 398. 13 full page plates, 80 text figs.
Vol. 2. Pp. 412. 10 full page plates, 75 text figs.
Vol. 3. Pp. 390. 13 full page plates, 51 text figs.

CONTENTS

PAGE

FISHER, R. A. (Cambridge). Statistical methods in genetics 1

MOURANT, A. E. (London), and WATKIN, I. MORGAN (Aberystwyth). Blood groups, anthropology and language in Wales and the Western Countries . 13

STANTON, W. R. (Hertford). Bolting, a vegetative variation in the potato 37

MULDAL, S. (Hertford). The chromosomes of the earthworms. I. The evolution of polyploidy . . 55

JINKS, J. L. (Birmingham). Heterocaryosis in wild *Penicillium* 77

REES, H. (Hertford and Birmingham). Asynapsis and spontaneous chromosome breakage in *Scilla* . . 89

DOWDESWELL, W. H. (Tiverton), and FORD, E. B. (Oxford). Geographical variation in the butterfly *Maniola jurtina* 99

RACE, R. R., and SANGER, R. (London). The inheritance of the *Duffy* blood groups 111

SÁNCHEZ-MONGE, E. (Zaragoza). The estimation of linkage with incomplete penetrance 121

COMMENT AND REVIEWS :

SAX, K., and LUIPPOLD, H. The effect of fractional X-ray dosage on the frequency of chromosome aberrations 127

LANE, G. R., on Sax and Luippold's fractionation experiment 131

DARLINGTON, C. D. on Cook's human fertility : the modern dilemma 132

DARLINGTON, C. D. on Scientific thought in the twentieth century 132

Books Received 133

GENETICAL SOCIETY OF GREAT BRITAIN : Abstracts of Papers 134